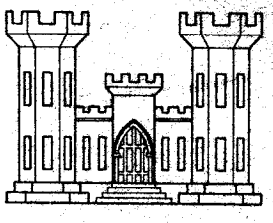

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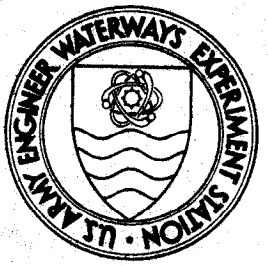

TECHNICAL REPORT D-78-23

CONSIDERATIONS IN CONDUCTING BIOASSAYS

by

David R. Rosenberger, Edward Long, Raymond Bogardus
Elaine Farbenbloom, Robert Hitch, Susan Hitch

Bioassay Laboratory
WAPORA, Inc.
Charleston, Illinois 61920

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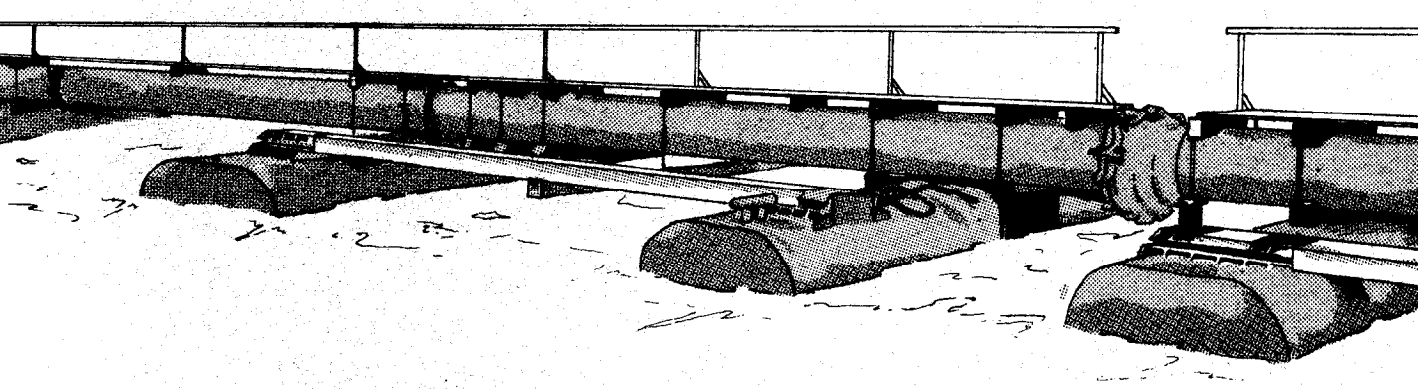

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1. The work reported herein was undertaken as Work Unit 1D02 of Task 1D, Effects of Dredging and Disposal on Aquatic Organisms, of the Corps of Engineers' Dredged Material Research Program. Task 1D was a part of the Environmental Impacts and Criteria Development Project (EICDP), which had a general objective of determining on a regional basis the direct and indirect effects on aquatic organisms due to dredging and disposal operations. The study reported herein was part of a series of research contracts developed to achieve the EICDP general objective.

2. This literature review is intended as an introductory overview of aquatic bioassays, with emphasis on dredged material bioassay techniques. It provides a general discussion of bioassay theory and practice, equipment, and selection of appropriate test species.

3. Species selection is discussed at length as it is one of the most important variables influencing the usefulness of bioassays for regulatory purposes. Similar criteria have been used by many authors in species selection with the basic consideration often being adaptability of the species to laboratory conditions and compatibility with testing procedures.

4. The information and discussion published in this report provide technical personnel unfamiliar with bioassays, managers, and administrators with a general introduction to the field of bioassay. This introduction to the bioassay literature will serve as a basis and guide in developing and implementing a regulatory program based on bioassays. It is expected that the information contained herein will be of significant value to those concerned with CE dredging material permit programs.

JOHN L. CANNON
Colonel, Corps of Engineers
Commander and Director

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Many types of tests can be considered as forms of bioassay. The broad range of substrate types, chemical composition, geographical locations, and equipment used in dredging adds to the complexity of formulating a single bioassay procedure applicable to all dredging programs. A discussion of the bioassay principles and techniques in the literature are presented to provide a background for an understanding of aquatic bioassay testing.

(Continued)

20. ABSTRACT (Continued).

Aquatic bioassays, which were developed from pharmacological drug testing techniques, often encounter complex toxicity problems. Two or more toxic compounds may be acting together or the toxicant concentration may fluctuate. The techniques used in aquatic testing may vary depending on the type of toxicant tested and the environmental parameters, such as temperature or dissolved oxygen, that need be controlled. Toxicant dosing equipment varies with the type of test species, source of water, and type of toxicant in question. Static bioassays are generally performed in glass jars or commercial aquarium tanks. Continuous-flow equipment consists of a reservoir of toxicant water which is metered into the test vessel. Extensive elaboration of the equipment has led to multiple toxicant delivery systems capable of providing a range of concentrations. The two most widely used delivery systems are the serial diluter and proportional diluter.

The selection of bioassay test species warrants careful consideration. The criteria for selection of species cited by most authors include the following not listed in order of importance: type of test; economic importance; ecological significance; geographical distribution; ease of capturing, handling, holding, and culturing; availability and local abundance; toxicity responsiveness; consistency of response to toxicity; and reproductive success under assay conditions.

The vast majority of species discussed in the bioassay literature are freshwater fish, primarily bluegill, rainbow trout, fathead minnow, goldfish, and largemouth bass. Infrequently used species generally did not fit all nine of the above selection criteria.

Recommended methods for capturing, handling, and maintaining many plants and animals for use in bioassays are presented in the report. Selection of wild species versus cultured species is the subject of continued debate. Wild specimens represent the fauna likely to be affected, but may be very difficult to maintain in the laboratory, while cultured specimens may be much more adaptable to bioassay testing.

SUMMARY

Pollutant materials become incorporated into the bottom substrates through the process of adsorption and sedimentation. Concern has developed in recent years over the possible deleterious effects to the water quality and aquatic organisms from the dredging and disposal of these contaminated sediments.

In response to Section 404 of Public Law (PL) 92-500 and Section 103 of PL 92-532, criteria have been formulated for determining the acceptability of dredged material disposal to the Nation's waters. The criteria allow, or in some cases require, bioassays to be performed on dredged material, but do not specify what type of study constitutes a bioassay.

A bioassay for the purpose of this report is defined as: a method of testing the potency or activity of a material (drug, naturally-occurring chemical, or pollutant) through the elicitation of a response (biochemical, physiological, or mortality) by a living organism, tissue, or cell. Behavioral responses are also considered in the report because of their significance in contributing to the ecological success of the species.

The screening of drugs for potency and specificity led to the development of classical bioassay principles and techniques, although many types of tests are presently considered as forms of bioassay. Aquatic bioassays are composed of three parts: stimulus, subject, and response. The application of these components to aquatic toxicity testing are complicated by the presence of multiple toxic elements more than one potentially toxic substance in many wastes and the presence of particulates which may sorb the pollutants, altering their toxicity. Calculation of the toxicity of these multiple systems has resulted in the development of mathematical formulae to estimate the concentrations which would cause mortality to the test species. Fluctuating concentrations of the toxicants have also been evaluated using mathematical formulations.

Numerous bioassay techniques have been developed for specific toxicant materials and test species. Variability of test results are

influenced by the chemical characteristics of the experimental water. Warm water decreased the survival time of fish exposed to heavy metals while cold water increased the susceptibility of fish to anesthetics. Effects of water hardness on survival times have also been reported.

There is general agreement that small organisms should be selected with at least ten used for each test. The organisms should be acclimated and tested in tanks constructed from nontoxic materials. Stainless steel or glass are most often recommended. Test water should be the same as the acclimation water and free from chemical or bacterial contaminants. The animals should be discarded if excessive loss occurs from disease or unexplained causes.

Static bioassay test chambers are almost universally constructed of either solid glass jars or commercial aquaria. Adaptation of the static tanks are reported for use with suspended sediments.

Continuous-flow bioassay equipment consists of an exposure vessel which receives a metered test material. The two most widely used multiple toxicant delivery systems are the serial and proportional diluters. The systems should require low maintenance and cease to deliver toxicant upon loss of dilution water.

In recent years there has been a tendency for toxicologists to incorporate on-site bioassay studies into their analysis of environmental toxicity. On-site studies allow for the natural variability of the toxicant in the receiving water. The equipment in simplest form consists of a holding box which is placed in the effluent water. Other systems consist of substrate material which is colonized by benthic organisms. A further advancement in on-site testing is to bring a portable bioassay laboratory to the test site.

A major requirement for conducting bioassay is an adequate supply of test organisms. Most authors agree that bioassay test species should possess certain qualities. The criteria cited by most authors for selection of test species include:

1. Type of test
2. Economic importance
3. Ecological significance

4. Geographical distribution
5. Ease of capturing, handling, holding, and culturing
6. Availability and local abundance
7. Toxicity responsiveness
8. Consistency of response to toxicity
9. Reproductive success under assay conditions

In tests evaluating site-specific impacts, species commonly found in the receiving water are often recommended. If a test is to be performed to establish standard concentration limits, common laboratory test organisms are recommended.

Organisms of economic importance should be considered as test species. The economically important freshwater species are almost entirely the Salmonid, Centrarchid, Cyprinid, and Catostomid fishes. Those species which are of economic importance also play a major role in the ecosystems to which they belong. Species which are important food chain species should also be considered as test species since they provide the biomass for higher organisms and they are known to bioconcentrate selected environmental contaminants. Test species which are healthy, unstressed and disease-free are essential in conducting a bioassay. Health problems may accentuate or mask the response of the organism, producing misleading results.

Species vary widely in their response to specific pollutants. The most meaningful ecological information is obtained by using a species of median toxicant sensitivity. Concurrently, the species should display a constant response to the test solution over time and between males and females.

Numerous authors noted some of the difficulties of using certain species as test organisms. The most common problems mentioned include: high mortalities, cannibalism, aggression, parasitism and feeding problems. Crustaceans commonly were reported to experience problems with cannibalism. Arthropods were very sensitive during molting.

Numerous species of plants and animals require special care in handling. Excessive handling can result in physiological stress which may influence the bioassay results. Methods and apparatus for culturing

some species of edible plants and animals have been available for many years. Others have developed only recently. The use of specimens taken from cultured stock ensures that the test organisms are free from pollution contamination and are descendants of the same genetic stock. Many of the economically important fish, crustaceans, and mollusks have been raised in the laboratory.

Wild-caught and cultured species can vary in their sensitivity to many environmental contaminants due to their differing genetic history. Species populations in a continually polluted stream are more highly tolerant to these contaminants than are species of an unpolluted source. Laboratory cultured specimens have been reported to develop an abnormal sensitivity to many pollutants. Selection of wild-caught versus cultured specimens should be judged by these factors.

PREFACE

The work described in this report was performed under Contract No. DACW 39-C-0134, titled "Considerations in Conducting Bioassays." The research was sponsored by the Office, Chief of Engineers (DAEN-CWO-M) under the civil works research program, "Dredged Material Research Program."

The research was conducted under the supervision of Mr. David R. Rosenberger, Manager, Bioassay Laboratory, WAPORA, Inc., Charleston, Illinois. Mr. Edward Long, Raymond Bogardus, Elaine Farbenbloom, Robert Hitch, and Susan Hitch assisted in the report preparation.

The contract was monitored by Dr. Paul Becker, assisted by Ms. Susan Palmer, under the direct supervision of Dr. John Keeley, Project Manager. General supervision was provided by Dr. John Harrison, Chief, Environmental Laboratory, U. S. Army Engineer Waterways Experiment Station (WES).

Director of WES during the period of the contract and the preparation of the report was COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

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CONVERSION FACTORS, U. S. CUSTOMARY TO METRIC (SI) UNITS OF MEASUREMENT

Units of measurement used in this report can be converted as follows:

Multiply	By	To Obtain
inches	25.4	millimetres
feet	0.3048	metres
gallons (U. S. liquid)	0.003785412	cubic metres

CONSIDERATIONS IN CONDUCTING BIOASSAYS

PART I: INTRODUCTION

Background

1. Industrialization and technical advancement have resulted in the production of new and exotic chemical compounds and increased amounts of waste products. Chemical control of undesirable plants and animals is now almost universal. Petrochemical wastes, mine-drainage waters, and industrial effluents are transported to the waterways through erosion, air deposition, and direct discharge. These pollutant materials become incorporated into the bottom substrates through the process of adsorption and sedimentation. Concern has developed in recent years over the possible deleterious effects to the water quality and aquatic organisms from the dredging and disposal of these contaminated sediments.

2. Contingent to the River and Harbor Act of 1970, the Environmental Protection Agency (EPA) formulated criteria for determining the acceptability of dredged material disposal to the nation's waters. However, a review of these early criteria by the Corps of Engineers[1] found that direct application to a particular dredging project was extremely difficult. A critical review of the literature relevant to the factors contained in the criteria provided insufficient data on which to base a decision as to the acceptability of dredged material for open-water disposal.

3. Bioassay is among the tests recommended in the criteria. Unlike phosphorus, sulfides, or others in the list, a bioassay is not a physical parameter which can be measured analytically, but is itself a technique for measuring the environmental effect of the other parameters. The criteria did not specify what physical and environmental conditions of dredged material would warrant the inclusion of bioassay tests as "appropriate and pertinent." Technical questions relevant to

implementation of regulatory bioassays include:

a. What type of biological test constitutes a bioassay?

b. Which species should be studied?

c. What types of biological responses should be observed?

d. What duration of time should the organisms be subjected to the dredged material?

e. What degree of biological response would determine the unacceptability of open-water disposal of dredged material?

State-of-the-Art

4. The preceeding questions do not imply a lack of knowledge for conducting aquatic toxicity bioassays. Extensive research in this field has resulted in the establishment of field and laboratory methods of conducting aquatic bioassays. These questions are, however, indicative of the "state-of-the-art" of conducting bioassays on dredged material. The general lack of published dredged material bioassay literature is evidence to this fact.

Source of Material

5. The material contained in this report is the result of an in-depth literature search and site visitation to recognized laboratories engaged in bioassay research where the problems and techniques of performing biological assays on dredged material were discussed. The equipment, testing procedures, and test species utilized for bioassay research were reviewed for their application to dredge material bioassay. Over 1500 research and literature review publications were analyzed for their contribution to this assessment. Additional unpublished literature was gathered from members of the scientific community engaged in bioassay and/or dredged material research.

Bioassay Definition

6. Pharmacologists have for many years used biological systems to test the therapeutic, effective dosage of drugs and the response

elicitated by a chemical. In more recent years, bioassays have been expanded to include environmental pollution biology. This expansion of use has often resulted in a misconception of what constitutes a bioassay. A bioassay, for the purpose of this report, is defined as a method of testing the potency or activity of a material (drug, naturally-occurring chemical, or pollutant) through the elicitation of a response (biochemical, physiological, or mortality) by a living organism, tissue, or cell.

7. Although not generally included in the definition, behavioral responses are also considered in this report because of their significance in contributing to the ecological success of the organism. Examples of behavioral responses include those which produce an avoidance reaction to a naturally-occurring chemical or pollutant; failure of the organisms to initiate or respond to mating behavioral activity; and changes in predator-prey interactions.

8. Beyond the scope of the above definition are those responses which produce a carcinogenic, mutagenic, or teratogenic reaction within an organism. Deletion of these responses from the scope of the definition should not be construed to imply a lack of their importance. To the contrary, their contribution to the ecological success of a species may prove as significant as an acute toxic mortality. Discussion of these topics in relation to dredged material must await development of an adequate data base.

Scope of Report

9. Many types of tests may be considered as forms of bioassay. Further, dredging activities encompass a wide range of substrate types, chemical composition, geographical locations, and equipment used. Selected environmental contaminants incorporated into dredged material may present special problems in bioassay investigations. Specialized techniques and equipment used in conjunction with these contaminants are presented. A discussion of bioassay principles and techniques are presented to provide an appreciation of the complexity of performing bioassays on dredged material.

PART II: PRINCIPLES OF AQUATIC BIOASSAY

Principles of Bioassay

Reason for study

10. The field of toxicology embraces a broad range of testing, interpretation, and application. The screening of drugs for potency and specificity has led to the development of classic bioassay principles and techniques.[2] A basic familiarity with the basic toxicological principles and methods of study is helpful for the interpretation and evaluation of bioassay results in aquatic toxicity testing.

Test for potency

11. Experimental pharmaceutical compounds undergo a series of bioassay tests to evaluate their biological potency. Reference compounds are selected as standards in the evaluation of potency. The experimental compound is tested against the standard in three types of studies.[3] The first is a measure of minimal potency. When given in low doses, the test compound must demonstrate an equivalent potency to that of the standard. A second study entails adjusting the dosage of the test compound to produce a submaximal response equivalent to the standard. Combination of the test results of these first two studies provides a range over which the drug may effectively be administered pharmaceutically (i.e., a minimum and maximum safe dosage). The dose-response curve is determined from the third study. The response observed at various dosage levels are plotted to evaluate the median effective dose. (The dosage level at which a 50 percent maximum response is observed.) The median effective dose curve is then used to evaluate the dosage schedule of the experimental drug. A modification of the latter type of test is the one most commonly used in aquatic toxicity testing.

Types of responses

12. Dose-response curves in pharmacological assay tests are based on two classical types of responses -- all-or-none and graded.[4] The all-or-none type is used to measure the threshold dose of the material

being tested. As implied by its name, the response observed is specific and distinct. Examples of all-or-none responses include mortality, avoidance, and nerve stimulation.

13. In all-or-none response tests, potency is inversely proportional to the smallest amount of drug that will cause the reaction. This level can be determined from curves based on a series of tests of separate subgroups exposed to different doses.[3,4]

14. In graded response, the amount of the reaction is a function of the amount of drug administered. When the response is plotted against the log-dose, a sigmoid curve is generally produced. Goldstein et al.[2] discuss exceptions to this rule. The area of the curve usually studied is the central linear portion of the graph. If two chemically similar drugs of different potency are administered in a series of tests, one may expect parallel log-dose response curves to the produced. This parallelism suggests that the mode of action of the drugs are similar. Nonparallel response curves indicate that the drugs affect different biological mechanisms or receptors (e.g., different enzymes or different sites on an enzyme).

Interpretation of results

15. The drug or toxicant undergoes a complex series of physical and chemical steps before its action is manifested as a biological response. If the test is not carefully designed, ambiguous or misleading results may develop.

16. The indiscriminate use of mathematical formulae, especially at the limits of their applicability, may yield improbable results. For example, in the formula: Concentration × Time = Constant, an infinitely large dose may be interpreted to produce an instantaneous response. However, this has been demonstrated not to be the case.[5]

17. The response that is being measured in the experiment must also be carefully defined. For example, in biological systems when a population of algae cells exhibits a decrease in growth rate after being exposed to a toxic substance, the decline might be due to the lowered mitotic rate of all the cells or to the death of some of the cells with a natural growth rate in the remaining cells. In the former,

the reaction is a graded response, but in the latter, an all-or-none response is indicated for each individual cell.

Application of results

18. Bioassay experimentation has lead to the development of some specialized forms of statistical treatments. Often two parameters of biological response are of interest: the median response and the threshold response. These responses can be expressed in either units of time or concentration of test chemical. The median response is of importance in representing the average response one may observe in a population exposed to the drug or toxic material. The threshold response signifies the point where an organism is first affected by the material. Threshold responses are most often used to determine a safe dose.

19. Gaddum[6,7] reviewed the state-of-the-art of bioassays, their mathematical relationship in pharmacology, and discussed the problems associated with determining a safe dose of toxicant. He felt that a safe dose should be established by setting arbitrary limits or application factors of 1/10 or 1/100 of a known threshold concentration. He recognized the variability of biological response and stated that these limits may not be applicable in cases of high biological variability. An alternate proposed method of determining a safe dose was to arbitrarily select three or six standard deviations below the median log-dose response concentration. The latter method was felt to be less costly and time consuming as both the median response and safe concentration could be ascertained from a single study.

20. Gaddum[7] believed that changes in such parameters as weight, blood pressure, and amount of food consumed may also be used as a measure of toxic effect. The use of the three to six standard deviations from the median dose necessary to affect this change may be more meaningful than acute mortality.

Statistical treatment

21. Statistical treatment of the median response is explored by Bliss[4] for time-mortality response curves. He describes the use of probit statistical analysis to convert the sigmoid time-response curve to a linear function. The tedious mathematical computations required

for this conversion have limited the usage of this technique. Most researchers have not been able to justify the time necessary to attain the precision possible using Bliss' methods.

22. Litchfield and Wilcoxon[8] proposed a graphic method for approximating the median effective dose. Data in its original form is plotted on probability graph paper and the confidence interval calculated by means of nomograph tables. Litchfield[9] presented a similar technique for calculating time-response curves. Recently, computer programming has offered more rapid usage of the probit computations.

23. Alternate methods to probit analysis are also available. Burdick[10] describes a graphical method for translating the sigmoid dose-response curve to a straight line. Harris[11] presents confidence limits and significance values for dose-response curves using a moving average-angle method. Further insight into the statistical treatment of bioassay data is available in the excellent work by Sprague.[12]

History of Aquatic Toxicity

General reviews

24. The development of aquatic bioassay began in the 1860's in England and in the 1910's in the United States. Penny and Adams (cited in Erichsen-Jones[13]) worked with the complex effluent from a dye factory and the individual components of the effluent. In the early 20th century in the United States Shelford and Wells (cited in Erichsen-Jones[13]) explored the suitability of different species for bioassay.

25. There have been several extensive general reviews of the bioassay literature produced over the years.[14-16] In 1964 Erichsen-Jones[13] reviewed the literature and techniques of bioassay up to the early 1960's. His work separated techniques by the type of substance being tested, e.g., metal salts, respiratory depressants, detergents, pesticides, and described equipment as well as results.

Specific aspects

26. Specific aspects of bioassay have also been reviewed over the years. Katz[14] covered the standard procedures of bioassay as they have

developed. Weiss[17,18] discussed the effects of organic pesticides on fish and Muirhead-Thomson[19] reviewed public health aspects of the use of pesticides. The toxic properties of metals in aquatic environments have also received widespread attention in review.[20-23] Doudoroff and Katz[24] reviewed bioassays of alkalis, acids, and inorganic gases, and Ball[25] reviewed fish bioassays and ammonia. The chemical aspects of the formulations reported in bioassay research have also been assessed.[26]

Water quality criteria

27. The problem of water quality criteria has been the subject of several papers.[27,28] In 1968 the Federal Water Pollution Control Administration[29] published Water Quality Criteria, which defined the standards and reviewed the earlier work on toxic substances and environmental variables. In 1956 the Ohio River Valley Water Sanitary Commission (ORSANCO) reviewed temperature limits, dissolved solids, chlorine, fluoride, and dissolved oxygen.[30] The direct and indirect effects of pH on European freshwater fish have been reviewed.[27,31] Lloyd[16] reviewed water quality problems for freshwater fisheries and recommended bioassays and field observation as the methods for achieving water quality standards.

28. The setting of criteria for estuarine organisms is a complex problem due to the physical diversity of the ecosystem as well as the diversity of life involved. In an estuary, invertebrates such as mussels and oysters are as important to sportsmen and commercial fishermen as are fish species.[32]

Scope of Aquatic Bioassay

Application of toxicological principles

29. Bioassay components. The application of toxicological principles to aquatic bioassay testing has been the subject of several reviews. Alderdice[33] noted that aquatic bioassays consist of three parts: 1) stimulus--such as drug, insecticide, or industrial waste; 2) subject--such as tissue, organ, or whole animal; and 3) subject response--such as death, change in weight, blood pressure, oxygen

consumption, or some other biological activity that has been evoked by the stimulus. The stimulus-response relationship for aquatic bioassay were reported to be of two separate types: lethal or acute, and sublethal or chronic.

30. Joint toxicity. Sprague[12] described a method for evaluating the joint toxicity of a mixture of compounds from their respective lethal threshold concentrations. Toxic units are presented which are equal to the actual concentration in the test solution divided by the lethal threshold concentration of the components of the solution. The toxic units of the respective components are added together, and if the total is greater than 1.0, the mixture is said to be lethal.

31. Field trials have also been made on predicting the toxicity of polluted rivers by adding toxic units. Sprague[12] reports that the toxic units may not be additive at low concentration, and the predicted total toxicity would be overestimated. Other cases of joint toxicity not covered by simple addition include those where two toxicants of the solution combine chemically.

32. Toxicant fluctuation. Fluctuating concentrations of effluent discharges present another special problem in aquatic bioassay testing.[34] Laboratory tests usually provide a constant dosage of test solution; whereas, in natural situations the concentration of contaminant and the volume of discharge varies from day to day or during the course of a day. Several techniques have been proposed for analyzing the toxicity of fluctuating effluents.

33. The first technique is merely to roughly associate the peaks of effluent discharges with observed fish mortality. A second technique involves calculating the survival times to concentrations of toxicants in laboratory studies. A curve of survival time versus exposure concentration is then produced. If environmental levels exceed those of the laboratory time-exposure curve, toxicity may be expected. A third method is based on the assumption that the rate of death is constant for a toxicant concentration. The rate of death per unit of time is then calculated at each level of toxicant. A peak of high toxicant discharge was then evaluated by plotting it as a graph of death versus

time. Counting the squares under the curve showed that fish died when accumulated death rates equalled unity. A fourth approach suggests that survival time may be estimated by calculating the average concentration of toxicant. If the average concentration is above lethal levels, fish may not be expected to survive. This approach may not be a true predictor of toxicity if exposure levels are at or near threshold levels.

Sublethal effects

34. One problem of acute toxicity testing is that the test cannot explain many of the ecological changes which occur in nature. Sublethal responses often occur when test organisms are exposed to concentrations much lower than necessary to cause an acute lethal response, but for much longer exposure periods. However, while certain sublethal changes, such as reproduction, are important, the ecological significance of other sublethal changes is highly questionable.[35] The physiological action of a toxicant is the key to predicting sublethal effects and depends on knowledge about the normal internal structure and functioning of the animal. There are several approaches that can be made. Histology is a very important tool for determining mode of action, but the primitive state of knowledge about aquatic organisms hampers this subject. There is no histological atlas for even a given species of fish. Physiology and biochemistry are also promising areas. Urine production, hematology, and blood chemistry have been tested in bioassay.

Variability of Aquatic Bioassay Testing

Variety of toxicants tested

35. Techniques for specific compounds. Aquatic toxicity investigations have produced numerous bioassay techniques for the study of specific types of compounds and test species. Because of these numerous techniques for different compounds and species, a single standardized bioassay technique has not been forthcoming.

36. Selected representatives of these specialized methodologies are presented in the following section to demonstrate the specialized problems which face the bioanalyst. Citation of specific techniques

does not constitute endorsement of the methods presented, but merely an attempt to provide total understanding of the bioassay literature.

37. Pulp mill waste. Warren and Doudoroff[36] discuss the development of methods for using bioassays in the control of pulp mill waste disposal. Discharges of these materials are of concern because they may create potentially toxic conditions for aquatic life, they may deplete the dissolved oxygen concentrations in the aquatic environment (possibly enhancing the toxicity problem), and/or they may promote the growth of slime or bacterial periphyton. The causative agent(s) responsible for any observed toxicity is largely unknown due to the complex composition of the effluents and the numerous steps in the manufacturing process. However, the complexity of pulp mill effluents has produced a wide range of reported toxicity characteristics that may fluctuate daily or even hourly. One explanation for the observed results is that potentially toxic, volatile materials may be lost to the atmosphere under laboratory conditions. Because of the potential loss of volatile solids, the high oxygen demand and potential changes in the chemical nature of pulp mill effluents, static bioassay evaluations may provide variable and not very meaningful results. Betts et al.[37] reviewed the test requirements of pulp mill effluent studies and concluded that continuous-flow studies were necessary to properly maintain test conditions and evaluate such discharges.

38. Betts et al.[37] attempted to evaluate the various components of the pulp mill waste treatment process. Miniature constant-flow screening was performed on Atlantic salmon (Salmo salar), followed by full-scale test conditions. Detailed descriptions are given for the experimental test tanks, cooling systems, stirrers, retention troughs, circulating pumps, temperature controls, and oxygen analyzers.

39. Oils and oil dispersants. Oil spills occurring along the coastlines of the continents of North America and Europe have led to the localized disruption of marine systems. Hazards to marine and estuarine fauna from the transport of oil, oil spills, and the dispersants used in cleaning up these oil spills have been studied using numerous bioassay techniques.

40. Crude and refined oils contain thousands of compounds with differing solubilities, volatilities, and toxicity. Toxicity data are available for various petroleum products, but usually the physical behavior of the oil in water mixture is not fully examined.

41. LaRoche et al.[38] conducted static bioassay investigations of the effects of oils and oil dispersant mixtures on the mummichog, a sandworm (Nereis vireus), and grass shrimp (Palaemonetes vulgaris). Homogenization of the solutions in water resulted in an increased toxicity to the species tested. The dispersants were found to vary in their primary affinity; some more soluble in seawater and others more soluble in the petroleum derivatives.

42. Lichatowich et al.[39] contend that crude and refined oils should be studied under continuous-flow conditions. They felt that the aeration required for static tests would accelerate the loss of volatile aromatics believed to be the prime contributors to the oil toxicity.

43. One of the most important considerations of the bioassay apparatus developed by Lichatowich et al.[39] was the size and shape of the test vessels, due to the changes in toxicity in shallow tanks or tanks with a large surface area. Two phases developed in the test aquaria. A slick containing most of the oil developed on the surface of the water, while some oil was present in the water column as soluble oil fractions or dispersed droplets.

44. Ecological behavior of the test fish influenced susceptability to the oil. Benthic fish, such as sculpin and flounder, remained on the tank bottoms and were least susceptible to the toxic effects; whereas pikefish and capelin positioned themselves close to the surface and were exposed to the more toxic effects of the oil. Those which remained in midtank demonstrated an intermediate sensitivity.

Variations of water quality

45. Temperature. One of the more important and most easily measurable factors that influence the outcome of a bioassay test is temperature. Because temperature can be readily controlled, more is known about this one factor than any other in the environment. Temperature changes can affect the activity, metabolism, and behavior of the

organism. The chemical and physical state of the toxicant, and the rate of uptake of the toxicant are also influenced by temperature.

46. It is generally recognized that there is a range of temperatures in which an organism can exist. The upper and lower lethal temperatures are dependent on the acclimation temperature. When the lethal temperature, both upper and lower, are put in the form of an area of response, it is called a zone of tolerance. Factors which affect these lethal temperatures include dissolved oxygen, pH, species, age, and size. Alderdice[40] exposed coho salmon to sodium pentachlorophenate, with a change of temperature, salinity, and dissolved oxygen. Analysis of the results indicated multiple interactions of the test variables.

47. The effect of temperature on the tolerance of fish to heavy metal poisoning has been reviewed.[41] He concluded, in general, that a decrease in temperature usually increases the survival time of fish in toxic solutions. Since low temperatures generally increase survival time of fish in toxic solutions, a lethal threshold concentration may not be applicable.

48. Durham[42] reviewed the influence of temperature on the toxicity of pesticides. He concluded that temperature interaction with biological systems are complex and no general rules can be applied.

49. Anesthetic gases dissolved in water are known to affect goldfish.[43] Fish acclimated to a range of temperatures received known concentrations of anesthetics. It was found that a rise in temperature resulted in a fall in anesthetic potency.

50. Relatively little information is available on the effect of heat upon microorganisms. Certain microorganisms do vary greatly in their temperature tolerance. In general, diatoms grow best between 15 and 40°C.[44] Protozoa are also able to live only within a rather narrow temperature range although the encysted or inactive stages extend this range considerably. Gradual acclimation also shows that microorganisms can adapt to higher temperatures if the changes are small and gradual.

51. Other water quality parameters. Other water quality parameters, such as pH, alkalinity, and hardness, have been shown to cause variability in the results of toxicity tests. Lloyd[41] cautions that

the extent of the difference in toxicity of heavy metals appears to vary for different levels of calcium concentrations. Some differences might be due to the decreasing solubility of the metals as the water becomes more alkaline, or the presence of calcium ions themselves reducing toxicity of the metal ions. There is the possibility that intracellular calcium content may also increase with the calcium content of the water, thereby reducing the toxicity of the heavy metals.

52. Zinc was found to be most toxic at a hardness of 50 ppm pH of 8 and least toxic at a hardness of 200 ppm and pH of 6. At a pH of 8 mechanical accumulation of zinc between gill filaments was noted followed by transfer to the epithelium and mucous. This may have caused the higher toxicity.

53. Water of low dissolved oxygen concentration has been found to increase the sensitivity of aquatic animals to toxicants. Hypothesis for this action is that respiratory rate increases with a lower oxygen content of the water, and the pumping of water over the gills increases the rate at which ions and molecules are adsorbed on the gills. Thus, the rate at which toxicants would enter a fish or aquatic animal would increase as the pumping rate of the water increases.[41]

54. Cairns and Schierer[45] observed the effects of periodic low oxygen upon the toxicity of various chemicals to aquatic organisms. Low oxygen concentrations were found to decrease the tolerance of bluegill to zinc chloride, naphthenic acid, and potassium cyanide, but not to potassium dichromate. The authors conclude that a series of threshold values exist for each chemical dependent on the dissolved oxygen content of the test water.

55. The factors affecting the resistance of rainbow trout to acid waters was investigated by Lloyd and Jordan.[46] Tolerance of acid waters was observed to increase with increased water hardness. Trout were also more resistant to acid drainage when 20 ppm CO_2 was added to the water.

56. Investigators rarely control the carbonate system and the pH system independently, even though the chemical ionic state of the metal is regulated. Roberts and Allen[47] give equations and procedures for

controlling total alkalinity and pH or total carbonate and pH in synthetic waters and in natural waters.

Current Proposed Standard Procedures

Sources of recommended methodologies

57. There are several sources of standard testing procedures for bioassay investigations. The more widely used procedures are those proposed by the American Society for Testing Materials (ASTM),[48] the Environmental Protection Agency,[49] and Standard Methods for the Examination of Water and Wastewater (Standard Methods).[50] Government agencies such as the Bureau of the Sport Fisheries and Wildlife,[51] the California State Water Pollution Control Board,[52] and ORSANCO[53] have published additional recommended methodologies. The EPA National Water Quality Laboratory[54,55] in Duluth, Minnesota, has published detailed methods for individual species.

58. The lack of a single uniform bioassay procedure to be used throughout the scientific community is due to several reasons. The most obvious reason is that individual authors differ in their opinion of the best research approach. Also, the testing of selected toxicants may require specialized procedures or conditions. In addition, the fish species selected for the test may place specific requirements on the size of the test tanks and rate of water replacement.

Fish

59. The ASTM[48] procedures specify that the fish preferably should be less than 8 cm long and weigh less than 5 g, and as a general rule that the larger fish in a set of tests in a tank should not be more than 1.5 times the length of the smallest one. Standard Methods[50] propose very similar standards, but define small fish as not more than 7.5 cm. The Fish Control Laboratory[51] uses fish between one-half and 2 g each. They recommend the range should not exceed 15 percent from the smallest to the largest fish. For testing the brook trout, EPA[54] recommends beginning with yearling fish with a total length of all the fish distributed between the tanks measured by a photographic method.

60. The EPA[55] gets their recommended fathead minnow, Pimephales promelas, eggs and larvae from stock cultures maintained in Duluth, Minnesota. At the beginning of the test 40 to 50 eggs or 1 to 5 day old larvae are distributed between the tanks. The fish are also measured by the photographic method at 30 to 60 days. Most authors are in agreement that at least ten individuals should be held at each concentration. The ASTM[48] standards recommend an acclimation time of 2 weeks, or up to one month if the fish is a cold water species. Standard Methods[50] recommends a ten-day acclimation period, while EPA[49] suggests at least two weeks.

Test tank

61. ASTM[48] suggested nontoxic tanks and other accessories be used in bioassay. It is important that the animal holding tanks are located away from any mechanical or physical disturbance, and they suggest a central drain so that wastes and feces can be removed. Standard Methods[50] mentioned that the test tanks should be large enough that the fish are not crowded. The EPA[55] regulations for the fathead minnow indicate the use of glass or stainless steel tanks with glass ends. For spawning purposes, there are two types of tanks which can be used. The first tank is 1 × 1 × 3 feet* with one of the ends of the tank screened off in a 1 × 1 × 1 foot chamber for separation of the young. The second type is 1 × 1 × 2 foot tank with separate tanks for the young totaling at least 1 cubic foot. The EPA[54] regulations for brook trout recommend 1.3 × 3 × 1 foot spawning tank with the water at least 1 foot deep, and tanks for rearing the juveniles 7 × 15 × 5 inches wide with a depth of 5 inches of water. The ASTM[48] recommend at least 6 inches of water, and Standard Methods[50] recommends the water be 15 cm deep. For their laboratory experiments, the Bureau of Sport Fisheries and Wildlife[51] uses almost exclusively 1-gallon pickle jars, which are discarded after use. They also have two types of containers for outdoor tests. One type is a wading pool with about a 7-foot diameter vinyl-lined tank, 2.5 feet

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* A table of factors for converting U. S. customary to metric (SI) units of measurement is presented on page 8.

deep, with a total capacity of 1000 gallons. Aquatic plants are added to these tanks before testing. The second type of test contains the Bureau of Sport Fisheries and Wildlife[51] use are 20-foot raceways for flowing water. These have disposal vinyl liners.

Test water

62. ASTM[48] recommends unchlorinated well water or a clean surface water source for the test diluents. Sprague[35] reminds the reader that even carbon-filtered tap water may be poisonous because small quantities of chlorine may remain. Synthetic waters are also available for both hard and soft freshwater and seawater. The Bureau of Sport Fisheries and Wildlife[51] uses reconstituted water for their laboratory tests. Standard Methods[50] recommends either unpolluted receiving water from upstream of the effluent or a reconstituted water for static tests. Filtered tapwater or natural water would also be acceptable in these tests. When a synthetic water is used, acclimation of the test fish is required for 10 to 30 days. EPA requirements for brook trout[54] and fathead minnow[55] suggest using water from a well or a spring or very clean surface water. As a very last resort, dechlorinated carbon-filtered tap water is acceptable when ultraviolet sterilization procedures are employed.

63. Water flow recommended by ASTM[48] is 1.44 liters of water per gram of fish per day. Aeration is considered necessary in the test tank. Standard Methods[50] and the Bureau of Sport Fisheries and Wildlife[51] suggested not over 1 g of fish per liter of water. For the EPA tests on the fathead minnow[55] and the brook trout,[54] respectively, the flow rates were recommended to be 6 and 10 tank volumes per 24 hours. Standard Methods[50] recommends withholding food for 48 hours before the test for short-term and static tests. The Bureau of Sport Fisheries and Wildlife[51] recommends withholding food for 96 hours before testing. They also recommend acclimating the fish to the test water without the toxicant for 24 hours prior to the test.

Chemical measurements

64. ASTM standards[48] recommend testing temperature, pH, and dissolved oxygen (DO) in the test tanks on the same schedule as the level

of toxicant is measured. A pH between 7.8 and 8.3 was recommended as a standard. Temperature for cold-water fish should be 15°C with 25°C ± 2°C recommended for warm-water fish. Dissolved oxygen should be held at saturation level. EPA requirements[54,55] suggest continuous recording of temperature, and DO measurements in at least one tank daily and in every tank at least once a week.

Disease

65. All independent sources promoting standard bioassays are agreed that disease and the percentage mortality in stock organisms should be kept to a minimum. ASTM[48] recommends that mortality actually be absent, or no greater than 5 percent during the 96 hours preceding the bioassay. The EPA fathead minnow[55] and brook trout[54] recommendations are that in case of an outbreak of a disease in the tanks, all the tanks should be treated for the disease at the same time and with the same level of treatment. Standard Methods[48] recommends not more than 10 percent mortality for 4 days before a test and no shipment of organisms should be accepted that show symptoms of disease, abnormal behavior, etc. The Bureau of Sport Fisheries and Wildlife[51] also recommends that when the organisms show a mortality greater than 10 percent prior to testing, none be used in the test.

Bioassay Equipment

Development of bioassay equipment

66. Aquatic bioassay equipment was first used in England during the 1860's. During the first decade of the twentieth century, English toxicology equipment was modified in many ways by American biologists.

67. Unfortunately, the influence of bioassay apparatus on the results obtained in toxicology tests was not critically evaluated until the second decade of the twentieth century. It was at this time that Belding[56] published his review of bioassay technology. The effect of such equipment characteristics as test container volume, construction material leachability, test water temperature, and test water oxygen content were all discussed in this early publication.

68. Before Belding's[56] research was published, there had been little interest in maintaining aquatic organisms in the laboratory for long periods of time. Researchers, for the most part, used nonquantitative field-sampling equipment to describe the general nature of polluted environments. Those investigators who did subject aquatic organisms to laboratory toxicological investigations were unable to determine chronic effects because no provisions were incorporated into the study for reducing the buildup of metabolic waste products. The test organisms themselves add toxic metabolic wastes to the control, test, and holding tanks by their normal physiological activities. Inadequate holding-tank filtration or the use of small holding-tank volumes allowed the buildup of the test organisms own harmful metabolities. The accumulation of nitrogenous wastes, especially ammonia, has been particularly troublesome.

69. Other shortcomings of early toxicological equipment have been determined since Belding's[56] study. The methods of maintaining constant toxicant concentrations for extended periods of time were not widely known during the early phase of toxicological studies. Early investigators were not equipped to maintain constant dosage levels of toxicants. The loss of test material in these early studies has been attributed to adsorption on the walls of the test chambers. Loss of toxicants also occurred through volatilization from open test tanks and natural chemical decomposition.

70. The maintenance of similar water conditions in the test chambers for the duration of an experiment was difficult for early investigators. Differences in water chemistry often occurred. The biological and chemical oxygen demand of a toxicant selectively reduced the dissolved oxygen levels at different exposure concentrations. Changes in pH and water hardness also were experienced through chemical reactions of the toxicant with the exposure dilution water. Variances in water chemistry during an experimental study may have caused altered chemical toxicity.

Modern dosing equipment

71. Static bioassay. The configuration and size of the equipment used in static or standing water bioassays has generally not changed

since the techniques were first developed. More information currently is known about the working volumes to be used with certain organisms and the types of materials recommended for construction of the holding tanks. Many tank construction materials have shown promise but have later been shown to have disagreeable effects on bioassay experiments. Fiberglass, polethylene, and polypropylene may absorb some organic toxicants causing an underestimation of the chemicals' toxic properties. Plastics and synthetic rubber products tend to release organics and plasticizers which may produce undesirable side effects to the test organism or toxicant. The material most often recommended for static bioassay tanks is glass. The tanks may be solid glass jars or aquarium tanks either purchased commercially or constructed to specification from double-strength plate glass cemented with a silicone rubber adhesive.

72. A variety of equipment has been used to maintain suspensions of sediment material in static bioassay experiments. Hubble and Reiff[57] successfully maintained a uniform suspension of particulate matter in a 30-gallon reservoir by an electric motor which raised and lowered perforated plates within the container. They were able to maintain 15 mg/l of suspended matter within 15 percent of its mean concentration for up to 7 days. Davis and Hidu[58] used a heavy-duty stirrer to keep sediment in suspension while a cup wheel delivered the suspended sediment to test aquaria. A more elaborate mechanism for maintaining suspending sediments was used by Davis.[59] The device was composed of polyethylene bottles placed on a revolving wheel. During toxicity experiments the wheel was continually turned to constantly agitate all the sediment to the same degree.

73. Continuous-flow bioassay. The equipment difficulties discussed thus far can be overcome or greatly lessened by constant replacement of the toxicant and water to which the organisms are exposed. Continuous-flow bioassay equipment has received much attention as to its design and function. While very elaborate designs have been reported in the literature, many do not possess functional reliability under continued operation.

74. All continuous-flow equipment consists of a reservoir of

exposure water which is metered into the test vessel. The toxicant may be contained in the water reservoir or may be metered into the test vessel separate from the diluent water. The toxicant can also be introduced on-line with the diluent water. Elaboration of this basic design provides for multiple dilution ratios and/or chemical modification of the test water.

75. The selection of a continuous-flow bioassay diluter should consider specific equipment features. Construction should be simple, requiring minimal effort and equipment expenditure. Calibration of the diluter to desired exposure concentrations must not be excessively complicated. Once calibrated, it should be capable of continued operation for extended periods of time. Maintenance needs to be minimal with only slight adjustments necessary to recalibrate the exposure concentration. When the diluter fails to operate, it must fail safely (i.e., toxicant is not allowed to flow into the test chambers in the absence of dilution water).

76. The simplest method of operating a continuous-flow bioassay is to decant a preset dilution of toxicant from a constant-head vessel such as a Mariotte bottle. Flow rates are determined by the size of the delivery tube and the height of the Mariotte bottle above the opening of the delivery tube. Variations in flow rates may result from rapid changes in atmospheric pressure or temperature.

77. Coler, et al.[60] varied the flow of toxicant from a constant-head dosing device by changing the angle of the tubing leading from a constant-head container. Their apparatus was, however, somewhat improved over earlier similar attempts in that a needle was placed on the tubing leading from the constant-head device in order to make the flow rate more precise. A more stationary mechanism for effectively changing the height of the toxicant water column was designed by Drammo and Kohlberg.[61] In this apparatus the toxicant flows from the constant-head storage vessel into a manifold which has pipes threaded into its base. To alter the rate of flow, the investigator screws the pipe in or out, changing its effective length.

78. A constant-head device designed by Lichatowick et al.[39] was

found to be capable of dispensing accurate doses of more viscous fluids. The apparatus consisted of a constant-head plexiglass box with a piece of elbow tubing protruding from the side. When a greater flow was desired, the tip of the elbow tubing was bent down to a lower level. Lichatowich and his coworkers found that this device was capable of dosing all but the largest particles (less than one-half inch in diameter) and was fairly accurate.

79. Anderson[62] combined the flows from two constant-head vessels to deliver seawater and chlorinated seawater to the eggs of Pleuronectes platessa.

80. Abrams[63] utilized a hypodermic syringe to dispense toxicants. The plunger of the syringe was driven by the flow of the dilution water. Design modifications by Stark[64] enabled delivery volumes to be maintained at five-percent error over a 24-week period.

81. Multiple toxicant delivery systems most widely used are of two types: serial diluters and proportional diluters. The common feature of both diluter systems is their capability of providing multiple toxicant concentrations to separate exposure chambers. The method of toxicant dilution is implied by their respective names.

82. The diluter described by Burke and Ferguson[65] exemplifies the design and operation of serial diluters. Water and toxicant are mixed in a series of chambers connected by drip lines. Successive dilution of the toxicant occurs in the connecting chambers. Aliquots of water from the chambers are delivered to the test aquaria. The water flow in the system is regulated by adjustment of the drip lines. The system is self-sufficient in operation, requiring no outside regulatory devices. Frequent measurements of the dilution concentrations are recommended to ensure that flow rates have not been reduced due to residue accumulated in the drip lines.

83. Proportional diluters developed by Mount and Brungs[66] operate on a sequential filling and emptying of water chambers. The water chambers are calibrated to contain a measured amount of water. Separate water chambers are provided for toxicant and diluent waters. The water chambers empty by siphon tubes activated by a venturi. Diluent and

toxicant waters are mixed in the siphon tubes and delivered to the respective test tanks. The cyclic action of the diluter is regulated by a solenoid valve connected to the inflow dilution water. The system is subject to electrical power failure and an alternate emergency power source is recommended with this installation. The system is widely used in institutions because of the ease in calibration and low maintenance requirements.

84. On-site bioassay techniques. In recent years there has been a tendency for toxicologists to incorporate on-site bioassay studies in their analysis of environmental toxicity. On-site bioassays allow for the natural variability of the toxicant receiving water, which is not possible in a laboratory study. Laboratory and on-site studies compliment each other in providing information on an environmental contaminant.

85. A simplistic form of in situ bioassay consists of maintaining test organisms in a holding box for observations. The holding box can be modified for specific research interests. A design for studying the effect of sediments upon benthic organisms was devised by Heitmuller, Del Wayne and Nimmo.[67] The structure consisted of a screen cage with a horizontal partition midway in the cage and an open bottom. To determine the effect of the sediment, organisms were placed below the partition. As a control, organisms were also placed in the top chamber removed from the sediment. Live cages made from braided nylon bags were used by Falk[68] to monitor the effects of an effluent upon pelagic organisms. In this design, light aeration was supplied to the test and control chambers by a small air pump.

86. Often benthic zoologists or phycologists allow naturally occurring fauna and flora to colonize on artificial substrate material. The number, size, or diversity of the populations which attach to the artificial substrates subjected to toxic chemicals are then compared to similar substrate in water above the effluent discharge.

87. The types of artificial substrates and the materials used to construct them are many and varied. The number, diversity, and per-day colonization rate of benthic macroinvertebrate populations taken by samplers made from 3M "conservation webbing" or from masonite plates

were compared by Dickson and Cairns.[69] Other macroinvertebrate substrates include hardware cloth baskets filled with stone (Wene and Eickliff[70]), hardware cloth and angle-iron cages (Henson[71]), and "Bar-B-Q" baskets filled with limestone (Mason et al.[72]). Kauss et al.[73] floated a polyethylene cylinder in shallow ocean water to test the toxicity of artificially produced oil spills on the various types of planktonic algae growing inside. Such conditions as algae density, temperature, dissolved oxygen, and pH of the water inside the cylinder were found to be very similar to the ocean conditions surrounding the cylinder. An artificial substrate for periphyton used by Dickman[74] consisted of agar-coated slides which could be floated in natural waters. Dickman[74] coated the slides with the bioassay treatment material, in this case germanium, and was thereby able to dispense with elaborate toxicant dosing mechanisms.

88. Another approach to conducting on-site biological monitoring is to actually bring a portable laboratory to the site to make closely controlled tests. The diluent waters are generally taken above the area where the industrial discharge is introduced. The toxicant can, likewise, be taken either directly from the industrial discharge or from a mixing zone within the receiving stream.

89. One of the most elaborate mobile bioassay laboratories was discussed by Zillich[75] in a review of on-site bioassays of combined chlorine residual. This apparatus--operated by the Michigan Water Resources Commission--is capable, through serial dilution, of running at many different dilutions (40 in the case of the Zillich study) with replication. The laboratory is also equipped to monitor the pH, dissolved oxygen, conductivity, and temperature of the effluents and of the polluted stream.

90. Exposure chamber modifications. Many modifications have been made on basic exposure tanks in an effort to more closely approximate the environmental conditions of organisms which have special requirements for survival. Planktonic organisms often fall out of suspension unless culture waters are circulated. Planktonic algae are often maintained in a flask or culture bottle with a magnetic stirrer inserted to

keep the medium in motion.[76,77] Other bioassay techniques for algae have been evaluated by Murray et al.[78]

91. Some animals require flowing water in order for their respiratory system to function properly. Benthic organisms requiring flowing water are generally exposed to toxicants with approximately the same type of equipment used in their culture. One such unit is an oval tank with the water constantly flowing in a circular direction.[79,80] Commercially prepared rectangular tanks which constantly force the water to the far side with a propeller on one end have recently become popular for tests on lotic benthos. The tank has a false floor through which the water can be drawn to be recirculated. The test organisms are generally situated on the top side of the false bottom. Both types of "artificial streams" have screens surrounding the water-impelling devices so that the test organisms will not be damaged.

92. Occasionally investigators have attempted to determine not only the effect of a toxicant on a single population of animals, but also the effect of biological influences such as parasitism and predation which would normally occur in that species' natural environment. One of the more closely controlled of such tests was devised by Goodyear.[81] Mosquitofish (Gambusia affinis) and largemouth bass (Micropterus salmoides) were placed in adjoining aquaria which were separated by a screened partition. The Gambusia could enter the largemouth bass tank where they would be subject to predation, but the largemouth bass were too large to enter the mosquitofish aquarium. Toxicant continually flowed into the bass aquarium and was allowed to pass into the Gambusia tank. Two types of controls were used for the test. One had a toxicant and no predation. The other contained no toxicant, but the predator was present. The relative number of Gambusia left in these variously treated aquaria after several days of exposure was felt to be a quantitative indication not only of the toxicant lethality but also of changes in predation due to the toxicant.

Sublethal tests

93. Environmental contaminants do not always elicit lethal effects when organisms are exposed to them. For this reason, researchers have

recently begun to investigate sublethal signs of stress in aquatic organisms. The numerous different types of sublethal parameters now monitored fall into the two major categories: either physiological or behavioral. Generally, larger organisms for which the physiological or behavioral indices are more easily monitored are used for these tests.

94. The amount of carbon dioxide evolved or the oxygen consumed by aquatic organisms has been monitored in much the same way by several toxicologists. An aquatic organism is placed in a sealed life-support system, the dissolved oxygen and carbon dioxide content of which is known. After a period of time, the concentrations of these two respiratory gases in the test water are again measured, and their depletions or increases are attributed to the metabolic functions of the experimental subject. Lee and Buzzell[82] measured the respiration rate of fish in a closed continuously flowing system with this technique. In an effort to measure the effect of various oxygen tensions on small benthic invertebrates, Gamble[83] bound the organisms with a hair inside a closed water trough. Securing the organisms in this manner obviated the need for other types of attachment substrate.

95. Monitoring of excretion ratio can be conducted with essentially the same support system as was used to measure respiratory gases. Lloyd and Orr,[84] for example, ran catheters from trout to an electrical contact. When the fish urinated, the electrical contact would close and a permanent recording device would be activated.

96. Equipment allowing the elicitation and measurement of behavioral responses has come into common usage only in recent years. Perhaps the most studied behavioral response is avoidance. Most avoidance tests have utilized large troughs which have inflow pipes for clean water and one or more discharge pipes. The amount of time that an aquatic organism spends near the inflow pipe carrying the toxicant is compared to the time that the organism spends in clean water areas of the trough. Most such troughs are large so as to allow the formation of distinct separation of clean and treated water. A much smaller chamber was designed by Jones[85] and has been used recently by Cook and Boyd[86] to study the avoidance reactions of the sand shrimp. The Jones[85]

avoidance apparatus consists of a glass cylinder secured in a ring clamp in the horizontal position. Toxicant enters one end of the cylinder at the same rate that clean water enters the other end. Both fluids continually drain through a hole in the center of the cylinder. Scherer and Nowak[87] described an electronic apparatus used to continuously record the movement of organisms during avoidance tests. The permanent chart recording of the movements of the test organism can be used to assess movement in avoiding the test material.

PART III: TEST ORGANISMS

Criteria for Selection of Test Organisms

97. "The major requirements for conducting bioassays are an adequate supply of test fish and dilution water, and laboratory facilities for holding fish and conducting the bioassays," (Henderson and Tarzwell[8]). Though some authors recommend fish for use in bioassays, they also agree that the forage species such as invertebrates which support these fishes are of sufficient importance to warrant selection in bioassay research.[89-91] Whether predatory or forage species are chosen for testing, the criteria cited by the authors for selection of individual species or phylogenetic groups are often numerous; differ from author to author; and vary with the type of test, test conditions, facilities available, and test objectives. The criteria cited by most authors for selection of test species are listed below in no particular order of importance.

a. Type of test.
b. Economic importance.
c. Ecological significance.
d. Geographic distribution.
e. Ease of capturing, handling, holding, and culturing.
f. Availability and local abundance.
g. Sensitivity to the toxicant.
h. Consistency of response to toxicant.
i. Reproductive success under assay conditions.

Type of test

98. The first criterion to be considered in the selection of a test species involves the consideration of the type of test to be performed (standardization, regional, or localized assessment, or behavioral response) and the substance being tested (turbidity, heavy metals, petroleum, pesticides). For example, in tests where the objective is to determine the lethality of a particular compound for establishing standardized concentration limits, only the very common laboratory species such as goldfish, guppy, or fathead minnow should be chosen. Mount[90]

recommended the goldfish as a possible aquatic equivalent to the laboratory white rat; although Katz[91] considered it "not highly regarded because of its tolerance." The EPA[92] in Duluth, Minnesota, has used the fathead minnow as a "white rat" in chronic tests and has developed a fathead minnow stock culture.

99. In tests that involve local toxicity or discharge problems from industrial effluents, pulp mills, dredging operations, or pesticide runoffs, those species which naturally occur in the body of water receiving the contaminant are often preferred. For example, bioassays involving contaminants discharged into cold northern streams should be conducted with cold-water organisms such as rainbow trout, walleye, northern pike, and cold-water invertebrates. Tests involving contaminants in temperate areas such as Chesapeake Bay should use endemic organisms exemplified by the striped bass, softshell clam, and blue crab. Becker et al.[93] recommended many suitable marine organisms according to eight geographic districts. Test data for laboratory species of goldfish and guppies may be of less value in a local toxicity problem than data collected with local or endemic species.

100. All species vary in their susceptibility to toxicants. Some are resistant to certain chemicals. For example, the quahog (Mercenaria mercenaria) is resistant to methoxychlor and malathion.[94] By careful examination of available literature, selection of resistant species can be avoided.

101. In short-term bioassays involving silt or turbidity, those species of snails, barnacles, and tubeworms which can seal themselves off from the surrounding water by means of an operculum or shell should be avoided. Numerous fishes and invertebrates appear to be suitable. The valuable oyster Crassostrea virginica was found to be very sensitive to turbidity and silt deposition and, therefore, may be suitable for use in turbidity bioassays. Certain other species, particularly those belonging to the families Libellulidae (skimmers), Chironomidae (midges), and the genera Simulium (black flies) and Chaoborus (phantom midge flies) tolerate high turbidity levels and would be less desirable selections for turbidity bioassays.

102. In avoidance tests, motile species which display an avoidance reaction should be selected. Many species of fishes have been used in avoidance tests, particularly species which are migratory and thus may be repelled from entering their natal streams by obnoxious or toxic substances.[91]

Economic importance

103. Many species of plants and animals are harvested in commercial or recreational activities due, in part, to their abundance, palatability, and availability. These harvests are of vast economic importance and need to be protected. Those species which satisfy this criterion of economic importance are often used in toxicity bioassays.[89-91]

104. The economically important freshwater species are almost entirely fishes, especially the Salmonids, Centrarchids, Cyprinids, and Catostomids. Various species of freshwater mussels and crayfish are of local economic importance in certain areas. In estuarine and marine waters, the spot, mullet, pinfish, striped bass, several salmonids, several decapod crustaceans, oysters, clams, and mussels are of economic value and have been used repeatedly in bioassays.[93]

Ecological significance

105. Those species which are of economic importance also play a major role in the ecosystems to which they belong due to their position as consumers and predators in food chains. Though most authors have only considered economically important animals, particularly fishes, for use in bioassays, some authors have recognized the significance of forage or food organisms in aquatic food webs and have selected them as test organisms in toxicity bioassays, since those primary food organisms which sustain the harvested crop must be protected.[90]

106. Diatoms have been selected as bioassay test organisms in marine and freshwater studies[95-97] because of their significance as food organisms and indicator species. The Florida Mangrove (Rhizophora mangle) is an important food source and nursery ground for many commercial fisheries, and has been used in bioassays.[98]

107. The saltwater shrimps Penaeus aztecus, P. duorarum, and Palaemonetes pugio are abundant and ecologically important along the

Gulf coast and have been used in bioassays.[99,100] Six species of freshwater crustaceans were selected for bioassays with herbicides in Missouri where they were considered to be important links in the food chains of fishes.[101] The ostracods are an important link in aquatic food chains, as they are widely distributed, omnivorous, scavenging crustaceans, and have been used in bioassays.[102,103] They occupy benthic and littoral niches in ponds, lakes, and slow moving streams where they filter-feed on bacteria, protozoans, algae and detritus.

108. Dragonfly nymphs have been subjected to DDT residue accumulation studies.[104] Many of the ecological and morphological characteristics of the insect suggest that they may be a major source of biological magnification in food chains. Dragonfly nymphs are described as a primary food source of carnivorous fishes and voracious predators of small insects. They pass a large amount of water over their gills, putting them in contact with pesticides in solution.

Geographic distribution

109. Data for organisms with widespread distribution are generally more valuable than those on species having restricted distributions. Most of the species recommended for use in bioassays by Doudoroff et al.,[89] Katz,[91] Henderson and Tarzwell,[88] Becker et al.,[93] Mount,[90] and Battelle-Columbus Laboratories[105] are widespread in distribution. Goldfish, bluegill, largemouth bass, fathead minnow, and rainbow trout have wide distribution throughout North America. They have also been selected often for use in bioassays.

Ease of capturing, handling, holding, and culturing

110. Keeping the test organisms healthy, unstressed, and disease-free is essential in conducting a bioassay. Those species which do not lend themselves to the procedures involved in capturing, handling, holding, and culturing should be avoided. Delicate fishes, and even some species which are quite hardy in nature, do not do well in captivity.[89]

111. The response of test organisms to toxicants varies with the relative well-being of the organism. Parasitism, injury, and other health problems may accentuate or mask the response of the organism to

the toxicant being studied, resulting in inconsistent or misleading data. Those chosen must also be capable of acclimating to a holding tank or aquarium and, if a chronic test is anticipated, must be culturable under controlled laboratory conditions.

112. Procedures for capturing and handling test fish for bioassays are summaried in several papers.[50,88,89,91,106] The authors of individual bioassay studies often include discussions of these methods in their text.

Availability and local abundance

113. Availability of an adequate supply of healthy fish of desirable uniform size may be the major factor in the selection of a test species.[88] Test species can be acquired from two major sources: collected from their natural habitat (wild-caught) or obtained from commercial bait shops, commercial hatcheries, government hatcheries, laboratory stock populations, or inbred, controlled pond populations.

114. Those species which are wild-caught must be available in large numbers year-round to ensure that an adequate number of healthy, uniformly sized individuals representing a natural male:female ratio are available from the population. There are species, readily obtainable in some seasons, that are impossible to obtain in others, or that may be available only in one stage of their life cycle. The seasonality of availability can be eliminated by maintenance of stock laboratory or pond populations.

Sensitivity to toxicants

115. The sensitivity of individual species to different chemicals varies greatly. Fishes that are tolerant of low oxygen conditions are not necessarily tolerant of toxic materials.[88] Some plants and animals are highly resistant to certain toxicants, while others may be extremely sensitive to the same toxicants. The sensitivity of some organisms changes with maturation from embryo to larva to adult and with variations in the physical-chemical conditions of the test water.

116. Those species which have median sensitivities to pollutants are most desirable for use as bioassay test organisms. Test species should not be resistant to the chemicals or conditions being tested.

Conversely, they should not be so sensitive that they die immediately upon contact with a very small amount of toxicant thus precluding a range of response being obtained.

117. The quahog (M. mercenaria) is resistant to methoxychlor and malathion[94] making it unsuitable for testing with these pesticides. The marine green alga Dunaliella tertiolecta and dinoflagellate Exuviella sp. are extremely resistant to lead and chromium.[107] Dunaliella tertiolecta was also most resistant to copper among nine species of phytoplankton,[108] and to urea herbicides among six species.[109] The marine clam Artica islandica is extremely resistant to hydrogen sulfide-rich solid waste eluates.[110] The periwinkle Littorina littorea was too resistant to oil to be used in bioassays; while the intertidal mollusc Patella vulgata was too sensitive.[111] Artemia salina larvae were resistant to copper and mercury in tests conducted in the United Kingdom.[112] The embryos of the sea urchin Strongylocentrotus purpuratus were selected for tests with oil products because of their noted sensitivity to pollutants but were found to be too sensitive to determine gradations of toxicity.[113]

Consistency of response to toxicity

118. Suitable test organisms should display consistent responses to toxic conditions to ensure uniformity in the data obtained. Those organisms which, due to morphologic or behavioral characteristics, respond variably among individual specimens should be avoided.

119. The degree of response consistency sometimes changes with variations in water conditions, age, sex, and life stage. The changes incurred by varying water conditions can be controlled and avoided; while those changes caused by biologic factors may not always be controllable. Sensitivity may also vary according to the environmental background of the specimens, i.e., the source from which the test organisms were acquired.

120. The greatest variations in toxicity response seem to occur among the arthropods. The arthropods not only differ in their responses with age and life stage (egg, larva, adult), but also differ during intermolt and molting stages. For example, several marine crustaceans

in Norway were found to be more resistant to surfactants during the intermolt stages.[114] The lobster Homarus americanus was shown to be most susceptible to crude oil during molting.[115]

121. Among inbred strains of platyfish (Xiphophorus maculatus), the males and females show differences in response to zinc.[116] Among the marine annelids, Capitella capitata displays a differential sex susceptibility to detergents.[117]

122. Fish sometimes demonstrate inconsistent differences in responses with age. Endrin is more toxic to adult Gasterosteus aculeatus (threespine stickleback) than to eggs and larvae, and to adult rainbow trout, chinook salmon, coho salmon, bluegill, mosquitofish, and guppies than to their respective embryos.[118] The fry of bluegills, green sunfish, smallmouth bass, lake chubsucker, and stoneroller, are more sensitive to several herbicides than their fertilized eggs.[119] Large specimens of the smallmouth bass, largemouth bass, bluegill, and black crappie are less sensitive to DDT than small specimens.[120] Older chinook salmon are less sensitive to DDT than the young.[121]

Reproductive success
under assay conditions

123. In chronic bioassays or those involving most of the species' life cycle, the ability of that organism to reproduce successfully in the laboratory is of importance. Suitable species are those that can be induced to spawn under laboratory conditions and demonstrate a relatively high survival of the fertilized embryos. It also is desirable that the larvae and immature stages develop to adults in satisfactory numbers.

124. The majority of the species selected by bioassay investigators are those which have previously been maintained under laboratory conditions following published cultural techniques of handling, maintenance, and care. Some species have been used more frequently that others; e.g., the bluegill (Lepomis macrochirus) appears in more published bioassay literature that does the orangespotted sunfish (Lepomis humilis). This does not necessarily imply that the bluegill is a better test species that the orangespotted sunfish. The dicotomy does serve to illustrate the widespread geographical distribution of the bluegill as

opposed to that of the orangespotted sunfish. Very few reports surveyed listed species which proved to be unsuitable for bioassays.

Species Most Commonly Selected

125. Table 1 lists the most commonly selected species from the reports surveyed which include 11 species of plants, 6 species of molluscs, 1 annelid, 13 crustaceans, 5 aquatic insects, and 31 fishes. All the species listed are of economic value or ecological significance, and most meet all nine selection criteria discussed above. The salmonid and centrarchid fishes comprise some of the most important game and commercial fishes in North America. The freshwater fishes as a group have been subjected to more bioassay tests with a greater variety of toxicants than any other group of organsims. All the decapod crustaceans (shrimps, crabs, lobsters, crayfish) and bivalve molluscs (clams, oysters, mussels) support multimillion dollar recreational and commercial industries in North America and abroad. The insects, annelids, and plants of Table 1 comprise important links in food chains since they are direct or indirect food sources for the economically important fishes, molluscs, and crustaceans.

126. A large number of marine and freshwater plants have been subjected to bioassays, but only 11 common species of planktonic diatoms and green algae have been used frequently (Table 1). The green alga Chlorella pyrenoidosa appeared most frequently in freshwater bioassays. The frequent appearance of the planktonic diatoms and green algae in bioassay experiments exemplifies their application as bioassay organisms. The small size, rapid reproductive rate, and extensive cultural techniques facilitate their use as test species. Patrick[122] discussed in detail the procedures for bioassays using diatoms.

127. Several species of marine molluscs have been selected often for use in bioassays. The mussel (Mytilus edulis), oyster (Crassostrea virginica and C. gigas), the quahog (Mercenaria mercenaria), and the soft-shell clam (Mya arenaria) have been used either as adults or as planktonic larvae (Table 1). Fewer freshwater molluscs have been used in bioassays. The snail Physa heterostropha was the most frequently

used freshwater mollusc encountered in the present survey.

128. A relatively small number of annelids have been reported for use in bioassays. The tubificid Limnodrilus hoffmeisteri is an important member of pond and stream benthic communities serving as a food source for fish populations. The frequency with which this organism appears in the literature indicates its general acceptance as a suitable test species. Several species of marine sandworm Nereis have been used in foreign and domestic research.

129. Among the freshwater fishes, five species, the bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), fathead minnow (Pimephales promelas), goldfish (Carassius auratus), and largemouth bass (Micropterus salmoides), appear to have been selected most often. These species are available throughout most of North America from bait shops, fish culturists, or hatcheries or are accessible from ponds, lakes, and streams. The above five species and many of the other species listed in Table 1 belong to the fish families Centrarchidae, Salmonidae, Cyprinidae, and Catostomidae. They were recommended for bioassay use by Doudoroff et al.[89] Katz[91] mentioned the bluegill, guppy, rainbow trout, fathead minnow, and goldfish as frequently used or standard fish. The above five species were among the 20 recommended for use in bioassay by Mount.[90]

130. The sheepshead minnow (Cyprinodon variegatus) and the mummichog (Fundulus heteroclitus), both common cyprinodonts, are among the most often selected marine/estuarine fishes. These two species are abundant in tidal ditches and estuarine marshes, and are easily maintained under laboratory conditions.

Species Infrequently Used

131. Table 2 illustrates the large number of species of plants and animals which have been used infrequently in bioassays. All species listed were either assayed in North America or are naturally available there. They appear to be suitable for use in bioassays. Most do not completely meet all nine selection criteria previously given. Some

have restricted geographic distribution or physiological and behavioral characteristics which present problems in assay conditions, and thus are slightly less desirable as test species. However, there are several notable exceptions to this rule which will be discussed below.

132. Numerous bacteria, yeasts, and marine fungi have been used in bioassays. There is a great amount of data in published reports and tests on isolating and culturing these organisms by microbiological techniques. However, most of this information deals with research efforts relative to disease transmission and human hygiene. Only 11 species were chosen as test organisms in the reports surveyed relative to dredging operations. The enteric bacterium Escherichia coli, is probably the most commonly selected form due to its reputation and importance as an indicator of water quality.

133. At least 70 species of freshwater and marine algae and 17 species of flowering plants have been used infrequently. As illustrated in Table 2, the vast majority of these species are planktonic, single-celled forms and few are macroscopic, benthic forms. Many of these planktonic organisms listed in the reports surveyed were not identified by species, indicating that the investigators experienced taxonomic problems with this group. That only a small number of benthic, macroscopic algae have been used is indicative of the problems inherent in laboratory culture of these plants. However, marine algae have been successfully cultured in several coastal laboratories and in the United Kingdom, and methods for culturing freshwater stream algae have been developed in several North American laboratories.

134. Corals, which are of great ecologic, geologic, and economic importance in tropical waters but which are relatively difficult to culture under laboratory conditions, did not appear as test organisms in the reports surveyed. Since these animals are very sensitive to siltation, they may be useful indicators of turbidity and siltation in the laboratory, provided methods for culturing them can be developed. Three Caribbean corals were maintained in the laboratory at the University of Georgia for a short time during feeding and respiration tests.[123]

135. Only two of the reports surveyed[124,125] include use of

platyhelminths in North America, both of which involved turbellarians. The turbellarian Polycelis nigra was selected for use in a bioassay with several toxicants in Wales.[126] Turbellarians such as the common Dugesia sp. are easily maintained and cultured under laboratory conditions.

136. The coot clam (Mulinia lateralis), which occurs in the coastal waters of the U. S., has proven to be an excellent laboratory animal and breeds successfully under laboratory conditions. Though this species has not yet been used extensively, its potential as a standard organism for bioassay and genetic research has been explored by the U. S. National Marine Fisheries Service in Connecticut.[127] It has been used successfully in temperature-tolerance tests in Chesapeake Bay.[128,129]

137. Many other species of molluscs not listed in either Tables 1 or 2 could be used in bioassays. For example, the freshwater mussels (Family Unionidae) selected in one study,[124] the marine shipworms (Family Teredinidae), and the marine scallops (Family Pectinidae) used in another[130] have been proven usable under laboratory conditions.

138. In addition to the tubificid and the nereid listed in Table 1, 16 other species of annelids were selected infrequently by domestic investigators. These included a leech, two tubificids, and many errant and sedentary polychaetes. Both larvae and adults have been used. Most of the annelids selected were polychaetes, including several sandworms (Nereis sp.) and tubeworms (serpulids) which occur in estuarine and marine waters of the U. S. The polychaete Capitella capitata, used in a California study,[131] has been cultured through succeeding generations and used in bioassays in France.[117]

139. Various species of acorn barnacles have been cultured in North America, the United Kingdom, and Scandinavia. A great deal of information on the biology and ecology of barnacles is available, due to their economic importance as fouling pests. In North America Balanus balanoides has been bioassayed often (Table 1) while B. eburneus, B. cariosus, and B. improvisus have been used less frequently (Table 2). Both the larvae and adults of these species have been assayed. The

larvae are easily cultured and are often more sensitive to toxicants than the adults.

140. The most economically important group of crustaceans is the decapods. In addition to the shrimps, lobster, and crab listed in Table 1, there are many freshwater crayfish, marine crabs, fiddler crabs, hermit crabs, and shrimps which are suitable as bioassay organisms. These animals have been used in both the larval and adult forms and generally are adaptable to laboratory conditions. All life cycle stages of Cancer magister have been assayed with pesticides at Oregon State University.[132] Carcinus maenas, which occurs in New England, has presented feeding problems to investigators in England.[133]

Test Species Previously Used in Turbidity Bioassays

141. Various species representing the bacteria, algae, molluscs, annelids, crustaceans, and fishes have been used in bioassays involving many turbidity-producing substances. A list of species and the type of substance they were exposed to is presented in Table 3. Test organisms were subjected to sludge, stream sediment eluates, paper and pulp mill effluents, silt, kaolin, paper and wood fibers, harbor sediments, ferric hydroxide flakes, and other substances.

142. The salmonids (e.g., Oncorhynchus kisutch, Salmo gairdneri), walleye (Stizostedion v. vitreum), minnows (Pimephales promelas, Notropis spp.), oysters (Crassostrea gigas, C. virginica), and quahogs (Mercenaria mercenaria) were selected most often. The larvae, embryos, juveniles, and adults of these species have been subjected to turbidity bioassays in one or more studies. The majority of the species listed in Table 3 are either fishes or benthic molluscs. Among the fishes selected, the salmonids and walleye were often used to illustrate avoidance among important, mobile animals. Also, these species naturally occur in geographic areas where pulp mill effluents and other turbid materials are commonly discharged into streams, thereby making them ideal choices as test organisms. Among the molluscs selected, the oysters, quahogs, and mussels are particularly sensitive to siltation due to the

nature of their filter-feeding apparatus. Since these animals occur naturally in bays, estuaries, and nearshore coastal areas subject to dredging and disposal operations; are sensitive to siltation; and are known to accumulate and concentrate heavy metals, they also make ideal test organisms.

Test Species Which Present Special Problems

143. Among the reports surveyed, numerous authors noted some of the difficulties or drawbacks in using certain species as test organisms (Table 4). Among these difficulties are problems which resulted in high mortalities, cannibalism, territoriality and aggression, susceptibility to parasitism, feeding problems, handling problems, and other special physiological and behavioral difficulties. Some of these species are also listed in Table 1, as "suitable (commonly used)," indicating that though these organisms have been selected often by a variety of researchers, they occasionally require special procedures to successfully implement a laboratory bioassay.

144. Large kelps such as Laminaria saccharina, which can attain a total length of 1 meter or more, can grow to be too large for use in laboratory conditions.[134]

145. Most benthic bivalve molluscs are filter-feeders and normally pump a large amount of water through their filtering apparatus to procure suspended food particles. In controlled laboratory conditions, a large amount of artificial or natural seawater supplied with suspended food must be provided to keep these animals alive for extended period of time. Many authors discussed mortality and feeding difficulties with Mytilus edulis.

146. The sensitivity of arthropods often changes from one life cycle stage to another. Most species are very sensitive during molting or ecdysis, prompting some authors to advise running bioassays on arthropods for all phases of life cycle or the most sensitive stages.[135] The major problem with some crustaceans is cannibalism, which makes necessary the providing of individual holding containers for each animal.

The time or point of death is often difficult to accurately determine among the small crustaceans, such as the amphipods and ostracods.

147. Among the problems encountered with the fishes, all three species of Alosa were susceptible to mortality during handling. Several fish species were susceptible to impaired health due to parasitism, including the goldfish, bluegill, mosquitofish, largemouth bass, and brook trout. Some authors experienced unexplained mortalities among test and control fishes. Some species tended to jump out of their test containers.

Test Species Unsuitable for Bioassays

148. Among the reports surveyed, only three species were considered by the authors to be unsatisfactory for use in bioassays. In a study with silt in South Dakota, tests with the drum (Aplodinotus grunniens) and the sauger (Stizostedion canadense) were unsuccessful because they did not adapt to aquarium conditions. The white crappie (Pomoxis annularis) was referred to as unsuitable under laboratory conditions.[136] However, since the crappie had been used successfully in pond studies in Illinois[137] and short-term aquarium studies in Iowa,[138] it must be considered as, perhaps, marginally suitable.

149. Studies with the marine lichen (Lichina pygmaea) in the United Kingdom[139] showed that such organisms, as a group, are not suitable for laboratory research, unless freshly collected specimens are used quickly for short-term studies.

150. The freshwater water strider (Gerris remigis) was found to be unsatisfactory in a study involving mirex in Mississippi.[140] Experimentation with this insect was terminated due to high mortalities in the control group.

Recommendations for Capturing, Handling and Maintaining Wild-Caught Organisms

151. The recommended methods for capturing, handling, transporting, holding, and maintaining numerous species of plants and animals for

use in bioassays are summarized and discussed in many published papers and texts. For example, the use of various capturing and holding techniques is included in Standard Methods[50] and discussed by several other authors.[88,89,141,142,143] Capturing and culturing techniques are also available for various freshwater and marine invertebrates, fish and algae.[144,145] The methods recommended by EPA for collecting bioassay test specimens and organisms for other laboratory analyses are described by Weber.[146]

152. Several physiological changes can result from handling stress[147] and subsequently influence the results of bioassay analyses. Among the reports surveyed, many authors mentioned problems with fishes, insects, crustaceans, and planktonic larvae being sensitive to handling, sometimes requiring termination of the tests.

153. Most of the reports surveyed which described research involving wild-caught organisms included a brief discussion of capture techniques and the equipment and methods used in holding and maintaining the organisms. The reports dealing with each species selected in the research surveyed are listed in Tables 1, 2, and 3. It is recommended that the appropriate reports be examined prior to a bioassay project to determine the acceptable and successful methods to be used with each species.

Recommendations for Culture of Test Organisms and Stock Populations

154. Methods and apparatus for culturing some species of edible plants and animals have been available for hundreds of years, while the techniques necessary to culture others have been developed only recently. When the purpose of culturing is to obtain organisms for use in bioassays, cultured stock populations provide the advantage of ensuring that the test organisms are free of pollutant contamination and are descendants of the same genetic stock. However, it should be cautioned that laboratory cultured organisms may have a different sensitivity than wild-caught or endemic species.

155. The majority of the freshwater and marine phytoplankton

utilized in bioassays were obtained from laboratory stock cultures. Among the reported bioassays involving use of freshwater species, many authors[148,149] obtained specimens from the Indiana University stock culture populations. Kauss et al.[151] used Bold's[150] basal medium in maintenance of their stock cultures. Most cultures were axenic and unialgal, though some investigators reported problems with bacterial contamination.

156. Bold[150] described in detail the procedures and apparatus for cultivation of algae and discussed special methods suitable for each algal group. The cultivation of marine and freshwater phytoplankton populations, particularly as food sources of cultured animals, has been described.[145,152-158] The green alga Selenastrum capricornutum which, along with Anabena flos-aquae and Microcystis aeruginosa, is recommended for use in bioassays, has been laboratory cultured according to the methods of the "Provisional Algal Assay Procedure" (PAAP).[159]

157. The methods of cultivating river periphyton communities have been developed and described.[160,161] The apparatus for culturing thermophilic stream algae under the conditions of natural stream flow have been developed by Sperling and Grumewald.[162]

158. Methods used in culturing and maintaining continuous supplies of marine diatom, dinoflagellate, and green phytoplankters for use in bioassays and similar experiments are available.[156,163-165]

159. Several macroscopic benthic algae have been laboratory-cultured for bioassays, mostly in the United Kingdom. Methods and apparatus for rearing marine benthic algae were summarized and described by Kornmann.[166] The red alga Callithamnion hookeri, a common British intertidal species, requires no medium enrichment, is easily cultured in small flasks, is morphologically simple, and grows rapidly.[167] Zoospores of Laminaria saccharina, a brown alga, were acquired from laboratory-reared adult plants and successfully tested with sewage, silt, and other pollutants.[168] This species and other members of the genus Laminaria are abundant subtidally in North America and are easily cultured, but present problems if they become too large for available facilities.[134]

Coelenterates

160. Many hydrozoans have been maintained in laboratories on a diet of brine shrimp (Artemia salina).[169] Among the species utilized, some have been wild-caught, while others have been cultured in the laboratory. The highly sensitive marine hydroid Eirene varidule was cultivated easily in Germany and proved to be suitable for marine bioassays.[170] The "sea nettle" (Chrysaora quinquecirrha) of Chesapeake Bay has been laboratory-cultured through the strobilae, ephyrae, and adult stages.[171] Adult medusae of Aurelia aurita developed from ephyrae within four months under controlled conditions in a chemically defined salt solution.[172] The methods used for raising Phialidium gregarium through its complete life cycle have been described.[173]

Bryozoans

161. Although few bryozoans were used in the bioassay reports surveyed, these animals are amenable to handling and culturing. Jebram[174] discussed the use of glass slides and coverslips in the cultivation of colonies of marine and estuarine species. The estuarine species Conopeum tenuissimum was also collected as larvae on glass slides and reared in a laboratory seawater system.[175] Freshwater ectoprocts were grown in aquaria in uncovered inverted petri dishes where they fed on natural suspended organic matter present in the water.[176] Several species of bryozoans have been raised on diets of phytoplankton.[169]

Rotifers

162. No rotifers were used as bioassay organisms in the reports surveyed. However, rotifers can be cultured in the laboratory. Species of Asplanchna were raised on Paramecium, which were fed cultures of bacteria.[177] Gilbert[178] described methods for initiating and maintaining cultures of Brachionus calyciflorus.

Annelids

163. A variety of oligochaetes and polychaetes have been used as bioassay organisms (Tables 1 and 2). Some of these species were acquired from laboratory stock cultures. The polychaete Capitella capitata was cultured through its entire life cycle, feeding upon dried Ulva every 5 days.[117] This species and other polychaetes have also been

raised on dried, resoaked Enteromorpha.[179] Hauenschild[169] lists many marine polychaetes as being laboratory-cultured. The relative rates of growth of annelid larvae fed various prepared and natural food materials were determined by Howie.[180] None of the authors who performed bioassays involving freshwater tubificids used specimens obtained from culture stocks.

Molluscs

164. Loosanoff and Davis[181] reviewed and described the recommended apparatus, conditions, and procedures for rearing eggs, larvae, and adults of many marine bivalve molluscs. Among the species they listed as having been successfully reared from fertilization to metamorphosis, the mussels (Mytilus edulis and Modiolus demissus), scallop (Aequipecten irradians), oysters (Ostrea edulis, Crassostrea virginica, and C. gigas), quahog, (Mercenaria mercenaria), surf clam (Spisula solidissima), and soft shell clam (Mya arenaria) have been used in bioassays in foreign and domestic laboratories. The rearing of marine bivalve larvae requires a large supply of unpolluted water, use of nontoxic pipes, filtration of the seawater, the addition of phytoplankton cultures as food, and apparatus to regulate water temperature. Bivalves can be conditioned to spawn out-of-season by manipulation of water temperature. Most cultured larvae were raised in natural seawater, though some authors[182,183] reported success with synthetic seawater. Most authors reported feeding the larvae protozoans and/or single-cell phytoplankton.

165. Woelke[184] proposed standard receiving water tests and criterion based on the use of 48-hour old Pacific oyster (Crassostrea gigas) embryos and described the equipment and facilities necessary for conducting such bioassays. Other reports which discuss rearing, supplemental feeding, bacterial flora, seawater sterilization, and production of clutch-free spat relative to laboratory analyses of oyster larvae are available.[185-187] The effects of microorganisms, organic substances, and algae cultured on treated sewage effluent on the feeding activities and growth rates of adult oysters have been described.[188-190]

166. The small coot clam, Mulinia lateralis, which was used occasionally in bioassays (see Table 2), has a short generation period,

a high reproductive rate, reasonable longevity, is easily cultured, and requires little space for rearing. This bivalve can be raised to metamorphosis in containers as small as one liter.[127] The soft-shell clam (Mya arenaria), often used as a bioassay organism, has been cultured and induced to spawn in prepared seawater.[191] Cyclic temperature fluctuations were used to induce ripe M. arenaria to spawn, and laboratory tests were undertaken to determine the basic environmental requirements of eggs and larvae of this species.[192] Stimuli used to induce Mytilus californianus to spawn were described by Young[193] and Carriker and Van Zandt.[194]

167. None of the reports involving bioassays with freshwater molluscs mentioned use of cultured specimens. Several freshwater river mussels, however, have been propagated successfully.[195]

Arthropods

168. Numerous crustaceans and some insects have been laboratory-reared for use in bioassays and other research. The methods for laboratory culture have been developed for some crustaceans because of their commercial value; while methods for rearing smaller crustaceans and insects for bioassay use were devised due to these animals' ecological significance, ease of handling, short life cycle, and ubiquity.

169. Several cladocerans, especially Daphnia magna, have been used extensively in bioassays because of their suitability as laboratory organisms.[196] The optimal photoperiod, temperature and feeding necessary to culture various species of Daphnia have been documented.[197-199] Field-collected Daphnia should be reared through at least two generations before commencing chronic toxicity tests.[200]

170. The brine shrimp (Artemia salina), often selected for use as a bioassay organism, are readily available as eggs from pet shops, easy to handle, usable in in-plant effluent surveys, and easily cultured. They can be sustained on daily rations of cultured yeasts. There is evidence that evaporation of the culture medium and replenishment with distilled water eliminated the repressive influence of accumulated secretions and excretions.[201] Several culture devices have been developed which facilitate continuous circulation and aeration of the culture

medium.[202] Currently, however, brine shrimp are not favored by EPA due to their general resistance and the fact that they are not typical or representative marine organisms.

Fishes

171. Many of the fishes often used in bioassays are acquired from cultured populations. These fishes have been reared in various aquaria, tanks, troughs, and ponds from fertilized eggs obtained from captured and cultured female adults. Several texts and publications contain general recommendations and methods for rearing fishes.[143,144,203-206]

172. Among the marine species Cyprinodon variegatus, Morone saxatilis, Clupea harengus, and several European species, including the plaice (Pleuronectes platessa), have been cultured from eggs for use in bioassays.[207,208] Northern anchovy larvae (Eugraulis mordax) have been successfully reared in laboratory containers where they were fed dinoflagellates, gastropod veligers, and brine shrimp nauplii.[209] Another oceanic species, the pilchard (Sardinia pilchardus) has been raised from larvae in circular tanks where they were fed natural offshore live plankton.[210] Several species of flatfish not used in the bioassays surveyed have been successfully reared.[211] Shelbourne[212] reviewed American and European methods of propagating marine fishes, experimental data, and recommended methods of rearing pelagic and demersal species.

173. Most of the North American salmonids, particularly the commercially valuable anadromous species, have been reared from eggs in large numbers in hatchery operations. Among the numerous bioassays conducted with salmonids, the majority of the specimens used were obtained as eggs or fry from cultured populations. Mason and Fessler[213] discussed use of a simple apparatus for incubating salmonid eggs at controlled levels of temperature, water flow, and oxygen. Culturing methods, spawning characteristics, and early life history of the commonly selected brook trout (Salvelinus fontinalis) are described.[214-216] The recommended bioassay procedures for the brook trout include information on all aspects of culturing this species.[217] The incubation period for trout eggs can be shortened by heating the water flowing through the incubators.[218] Poon and Johnson[219] recommended procedures for transporting

salmonid eggs, important in bioassays where experimentation is carried out at a location distant from the hatchery where eggs are acquired. A compact recirculation system can be used to rear trout with low mortality and satisfactory growth rates.[220] Burrows and Combs[221] reviewed the use of controlled environments for propagation of salmon, and Burrows and Chenoweth[222] described rectangular circulating ponds as having higher operational efficiency than other types of ponds for rearing salmon. Brown trout (Salmo trutta) have been raised in glass aquaria during growth rate research.[223] Nutritional data for raising fingerling salmon are provided by Fowler et al.[224] Most salmonids raised for use in bioassays were fed prepared dry foods and chows, which has been found to contain DDT, chopped liver, or moist pellets.[225] Thus, DDT and residues have been detected in various salmonids acquired from hatcheries and commercial suppliers, resulting in 30 to 90 percent mortalities during the days following the swim-up stage.

174. Cultivated aquarium goldfish (Carassius auratus) are quite hardy, do well in aquaria, tanks, bowls, and ponds at room temperature; and breed successfully at one year of age.[226] Another aquarium species, the flagfish (Jordanella floridae), is readily available through aquarium shops, easily maintained, and reared. "Spawning mops" made of nylon yarn can be used to collect the fish's eggs.[227] Adult flagfish over 2.5 cm in length should be fed frozen adult brine shrimp ad libitum twice daily, supplemented by one daily feeding with a high-quality, fine-granule dry trout food. Only F_1 or later generations should be used in bioassay testing.[228] The inbred platyfish (Xiphophorus maculatus) has been suggested as an animal that can be used for studies of aquatic toxicants, and has been recommended as a bioassay animal.[229]

175. The fathead minnow has been cultured often for the purpose of providing a continuous supply of eggs or fish of known age, and raised under identical conditions for use in bioassays. Procedures for establishing and operating a fathead minnow stock culture are recommended by EPA.[92]

176. Guppies (Lebistes reticulatus) can be maintained and cultured easily in the laboratory where the females produce broods about

once per month. The adults are fed prepared dry foods, brine shrimp, or other common aquarium fish foods.[229,230]

177. Although the warmouth (Lepomis gulosus) was not used in the reports surveyed, it is suitable as it will nest and spawn in laboratory aquaria.[231]

178. The bluegill (Lepomis macrochirus) can be raised in the laboratory for use in bioassays. Procedures for maintaining adults, gathering eggs, incubation, and rearing of young are described by the EPA.[232]

179. Most of the fishes listed in Table 1 and some of those listed in Table 2 have been cultured. Among those species cultured for use in bioassays, besides those discussed immediately above, are the walleye (Stizostedion v. vitreum), sucker (Catostomus commersoni), lake chubsucker (Erimyzon sucetta), stoneroller (Campostoma anomalum), largemouth bass (Micropterus salmoides), smallmouth bass (M. dolomieui), green sunfish (Lepomis cyanellus), redear sunfish (L. microlophus), pumpkinseed (L. gibbosus), channel catfish (Ictalurus punctatus), black catfish (I. melas), yellow perch (Perca flavescens), carp (Cyprinus carpio), striped bass (Morone saxatilis), northern pike (Esox lucius), golden shiner (Notemigonus crysoleucas), and bluntnose shiner (Notropis heterolepis). Yellow perch often do not accept trout food pellets but accept mixtures of trout food and gound beef liver.[233] The white sucker has spawned and been reared in the laboratory being fed brine shrimp when young and trout food when older.[234] Apparatus and methods for rearing carp and northern pike were discussed by Blaylock and Griffith[235] and Adelman and Smith,[236] respectively.

180. In bioassays involving use of marine organisms, artificial rather than natural seawater may be used to minimize biological effects and to provide a reproducible solution of known composition. Several commercial salt preparations are available and usually result in successful bioassays. Kester et al.[237] provided a method for preparing artificial seawater. The design and operation of open seawater systems using natural seawater have been reviewed and described.[238-240] A closed recirculated seawater system was described by Parisot.[241]

181. Apparatus for rearing aquatic plants and animals is

described by Sudia[242] and Whitford and Dillard.[243] This equipment provides for controlling water flow, photoperiod, and other critical parameters.

Wild-Caught Versus Stock-Cultured Test Organisms

182. As discussed above, many test species have been acquired as wild specimens with nets, seines, traps, etc. and have also been cultured in the laboratory. Other species have only been obtained from wild populations and a few (mostly exotic tropical fishes) have been acquired only as inbred cultured organisms. It is possible that wild-caught and cultured specimens of one species may differ in toxicity responses due to their environmental background or genetic makeup. In bioassays of zinc and copper with the Atlantic salmon (Salmo salar) collected from a river and from a federal hatchery, no differences in avoidance response were apparent in fish from the two sources.[244] However, Irwin[245] observed that a species population in a continually polluted stream will be composed of fish with higher resistance than the original population or one collected from an unpolluted source. Some species acquire resistance to pollutants when living in affected streams.[246] All specimens used in a single test should be acquired from the same source.

183. Since samples of a single species of fish from two or more sources may differ greatly in their response to a given chemical, Lennon[247] strongly urged that fish toxicologists follow the lead of mammalian toxicologists and pharmacologists and develop standard reference strains of fish for reproducible and comparable bioassays. Many species, particularly freshwater fishes, have been repeatedly cultured in inbred stocks in attempts to eliminate from laboratory experiments the natural biological variability among members of a population and to insure that the test organisms have spent their lives in the same controlled, reproducible medium. Acclimation or immunity to toxicants is also prohibited via the culturing method. However, there is the hazard that the cultured organisms will acclimate to unnatural

laboratory conditions or conditions which do not undergo natural diurnal, monthly, or seasonal cycles, or that the inbred population will be selective in succeeding generations for genetic factors not normally selected for; thus leading to a population gene pool unlike that of a natural population. Therefore, toxicity response may differ between wild-caught and cultured specimens, and data collected in laboratory tests with laboratory-reared animals may not be applicable to natural populations living in streams, ponds, and oceans.

Recommended Test Species

184. Plant and animal species often recommended and chosen for use in bioassays are those which meet most or all of the nine criteria listed earlier. The selection criteria may also include uniformity of size, small or practical size, and availability in a healthy disease-free condition. Several recent publications have included lists of recommended test organisms, particularly fishes, in discussions of bioassay methods.

185. Although any fish species which suits the purpose of the investigation may be used, Doudoroff et al.,[89] recommended selection of fish species belonging to any of the following widely distributed and important families, unless there is sufficient reason for making a different choice:

Centrarchidae (sunfishes, basses, crappies)
Salmonidae (trouts, charrs, salmons)
Cyprinidae (true minnows), exclusive of carp and goldfish
Catostomidae (suckers)

186. Henderson and Tarzwell[88] discussed fish species which have been used successfully in bioassays. The authors feel that the fish should be readily available, adaptable to laboratory conditions, and should be of small size. They describe the fathead minnow as an excellent choice based on their selection criteria. Other fish species are also described which have been used for bioassays.

187. Henderson and Pickering[248] suggest the use of the guppy

(Lebestes reticulatus), mosquitofish (Gambusia affinis), goldfish (Carassis auratus), fathead minnow (Pimephales promelas), and bluegill (Lepomis macrochirus) in warm-water bioassay studies. While other species of sunfish, bass, and minnows may also be used, they caution the use of certain minnows (Notropis) as they are difficult to maintain in the laboratory. Although cold-water species as generally not suitable for warm-water studies because of their temperature requirements, trout can be used if water temperature is carefully controlled.[248]

188. Irwin[245] described the techniques for capture, transfer, transport, and feeding of fish as being critical to the success of a bioassay, and discussed several problems encountered in using various species. The white bass (Morone chrysops), for example, went into tetany when placed in shallow water, and the gizzard shad (Dorosoma cepedianum) leaped out of the tanks if not handled properly. He listed several criteria for selecting a test fish: availability in large numbers, proper (small) size, health, adaptability to laboratory life, metabolic rate, acceptance of food, and no tendency to jump out of test containers.[245] He also presented a ranking 57 species of freshwater fishes as to their suitability as test animals in oil refinery waste bioassays (see Table 5).

189. Other researchers have proposed lists of suitable test species.[90,142,249] A comparison of these lists are presented in Table 6 along with a list of commonly selected species compiled from a recent survey of bioassay research.[105] The list of commonly selected species in the Battelle survey is nearly identical to that compiled in the present study (Table 1).

190. Mount[90] compiled two lists of fishes for use in bioassay research based on the recommendations of the staff at the EPA Laboratory, Duluth, Minnesota, and recommendations of U. S. authorities (see Table 6).. Those species included in the second list (for selected pollutants) were described as having limited distribution or having special requirements but also suitable for study in regard to certain kinds of pollution. The goldfish was selected as the species most meeting the description of the piscine equivalent of the laboratory white rat.

191. Among numerous groups and species studied, Tarzwell[250] selected four species for use in bioassays: the fathead minnow (Pimephales promelas) for freshwater tests; the mummichog (Fundulus heteroclitus), eastern oyster larvae (Crassostrea virginica), and brine shrimp larvae (Artemia salina) for marine tests.

192. Finally, Becker et al.[93] recommended use of many marine vertebrates, invertebrates, and plants in oil and dispersant bioassays in eight geographic areas of the United States. Many of the species selected were recommended for more than one geographic region and many are of some economic value or local ecologic significance. Other selection criteria used were vulnerability to marine pollutants such as oil slicks in a critical life stage, availability and ease of collection, ease of rearing and maintaining in the laboratory, occurrence in intertidal or estuarine areas, and existing knowledge on ecological requirements. The species included in the lists of key reference biota discussed above are compiled in Table 7.

193. Based on the information gathered in this survey on test species suitability and on the recommendations and conclusions of reports discussed above, the authors recommended selection of test species from the lists presented in Tables 1 and 7. Selection of these species is contingent upon local availability.

194. In addition to those species listed in Table 1, various other organisms appear to be suitable or have been recommended by authoritative toxicologists. These include the algae Anabaena flos-aquaea, Microcystis aeruginosa, and Selenastrum capricornutum; the molluscs Mulinia lateralis, and Rangia cuneata; the crustaceans Acartia tonsa, Asellus militarus, Cancer magister, Carcinus maenas, Gammarus oceanicus, Orconectes nais, Procamburus clarki; the insects Classenia sabulosa, Ephemera simulans; the fishes Clupea harengus, Jordanella floridae, Xiphophorus maculatus. Numerous other plants and animals have been used successfully in laboratory and field bioassays and are suitable for use, but due to their limited distribution or other factors have not been used extensively. If the investigators are familiar with these less frequently used species and these species have proven to be

suitable for bioassay use, then they should be selected if the more commonly used species are not available or if the use of the commonly used species will not provide the data needed in the research being conducted. That is, any species which suits the purpose of the anticipated research should be used; however, those species which have been used often before, and for which there are previously acquired data, should be given first consideration.

PART IV: DREDGED MATERIAL BIOASSAY DEVELOPMENT

195. Prior to about 1970, the only Federal regulatory control of dredging was under the River and Harbor Act of 1899, which required that a permit be obtained from the Corps of Engineers for virtually all work in navigable waters which might affect navigation. In the 1960's public awareness and concern over environmental problems increased, with the greatest initial concern over dredged material disposal in the Great Lakes region. In the late 1960's at the request of the Federal Water Quality Administration (FWQA), the Corps of Engineers Buffalo District initiated studies on the chemical characteristics of selected Great Lakes harbors. The earliest guidelines proposed for the regulation of dredging and dredged material disposal based on results of the Great Lakes Survey, were promulgated in 1971 by the EPA. That year the EPA and the Corps of Engineers issued Engineering Circular 1165-2-97 based on EPA's memo which stated that under Section 10 of the 1899 Refuse Act the dredged material disposal "bulk sediment" chemical criteria formulated by the EPA, commonly called "the Jensen Criteria," should be applicable to sediments dredged from all U. S. waters.

196. Regulation of dredged material disposal under the 1899 Refuse Act came to an end in 1972. That year Congress passed the Marine Protection, Research and Sanctuaries Act of 1972 (P.L. 93-532), and the Federal Water Pollution Control Act Amendments of 1972 (P.L. 92-500). It was then mandated that the Corps of Engineers, using criteria and guidelines developed by EPA in consultation and conjunction, respectively, with the Corps, use Section 103, P.L. 92-532 to regulate dredged material disposal in marine waters, and Section 404, P.L. 92-500 to regulate dredged material and fill material disposal in freshwater and fill material disposal in marine waters.

197. Testing of dredged material prior to disposal is required both by P.L. 92-500 and 92-532 to evaluate the potential environmental impact of disposal operations. As presently constituted, the guidelines for implementing Section 404 of the Federal Water Pollution Control Act Ammendments of 1972 (P.L. 92-500) call for evaluation of the physical

and chemical-biological interactive effects of dredged material disposal, and a site selection evaluation. Tests which may be conducted to evaluate chemical-biological interactive effects include the elutriate test, water column and benthic bioassays.

198. The criteria for ocean disposal of dredged material under Public Law 92-532 take into account provisions of the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter. The Convention, of which the U. S. is a signatory party, was held in November 1972 and became binding international law on 31 July 1975. The Convention bans the ocean dumping of materials containing other than traces of a long list of compounds. Compounds on the prohibited list are considered to be present in trace quantities when the dumping of dredged sediments containing these contaminants will not cause significant undesirable effects.

199. The potential for undesirable impacts of dredging and dredged material disposal are assessed under the ocean dumping criteria by means of liquid, suspended particulate, and solid phase bioassays. The bioassays required by the criteria make possible the direct assessment of potential environmental effects on aquatic organisms during and after the dredged material disposal operations. This direct determination of the biological effects is much more accurate than attempting to infer biological effects from chemical constituent concentrations in water or sediment. Bioassays that reflect conditions at the disposal site provide a solid technical basis upon which regulatory decisions can be defensibly implemented.

200. It is officially recognized that the present state-of-the-art does not allow the development of "final" criteria, test procedures, and similar decision-making guidance for either Section 103 of Public Law 92-532 or Section 404 of Public Law 92-500. Provisions are therefore present in both laws whereby periodic review and revision or updating are possible as more is learned through both research and experience. Research is continuing to close knowledge gaps and develop better disposal criteria and guidelines. Environmental research relative to dredging and disposal is being closely coordinated between the Corps and EPA

under the auspices of the EPA/CE Technical Committee on Criteria for Dredged and Fill Material. This joint technical committee was formed to focus attention on research coordination and needs and is staffed with senior level scientists from both agencies with broad knowledge and responsibilities for research activities. The Committee has published a technical procedures implementation manual for the ocean dumping criteria,[831] and will prepare a similar manual for implementation of the final regulatory guidelines on Section 404 of P.L. 92-500.

201. Bioassay studies conducted with dredged material have been limited in number and scope. Two general types of dredged material bioassays are of interest; those addressing water column effects and those concerned with effects upon benthic organisms.

202. Workers conducting water column bioassays have investigated a limited number of organisms and methods of bioassay water and sediment preparation. Emerson[832] used benthic polychaetes and sediment extracts of varying sediment/water ratios. Hoss et al.[833] used sediment extracts made from seawater and marine sediments to determine the effects of soluble compounds released from the sediments on larval fish. Both found that the sediment/water ratio used in preparing the extract was important, and that extracts of some sediments were toxic to some organisms after prolonged exposure to high concentrations. Both liquid and suspended particulate phases of Bailey Creek, Virginia, sediments were toxic to Daphnia at 100 percent concentration; that is, when no allowance was made for the mixing and dilution required by the guidelines.[834] The soluble and particulate phases of Perth Amboy and Bay Ridge, New York, sediments also showed some toxicity to the estuarine copepod Acartia tonsa, grass shrimp (Palaemonetes sp.) larvae, and adult opossum shrimp (Mysidopsis bahia) when tested at 100 percent concentration.[834] No toxicity was observed when the bioassays were conducted with elutriate preparations mixed with disposal site water at concentrations representive of observed mixing conditions, as is required by the criteria and guidelines. Thus it can be seen that the current bioassay techniques tend to overestimate sediment pollution potential.

203. The short-term impact of dredging and disposal operations

upon aquatic test organisms has generally been negligible. Shuba et al.[835] investigated the effect of the liquid phase of dredged material upon various aquatic organisms. An inhibitory effect on algal growth was found only in the liquid phase of sediments from Mobile Bay, Alabama. The inhibition was noted under "worst case" conditions when the elutriate was used full strength with no dilution by disposal site water and is indicative of conditions in a barge, hopper, or pipeline rather than the water column immediately after disposal. Algae showed promise as a group of organisms for use in regulatory testing. Bacteria and protozoans were found unacceptable for use in dredged material bioassays because of poor response to test solutions. Development of an algal bioassay was continued by Shuba et al.[834] and refined to the point where it could be incorporated into dredged material evaluation procedures.[831, 836]

204. Lee et al.[837] conducted static standard elutriate bioassays and found the grass shrimp (P. pugio) sensitive to 96-hr continuous exposure to high concentrations of elutriates prepared from some sediments.

205. Lee et al.[838] reported that laboratory bioassays with unfiltered elutriates (suspended particulate phase) have shown very little toxicity to aquatic organisms during worst case simulations of disposal in open waters. Daphnia or grass shrimp (Palaemonetes) survival for 96 hours in the laboratory without significant mortality was observed in the equivalent of a settled discharge from a dredging pump or hopper and barge bin.[838]

206. Research results have generally shown that the soluble and particulate phases of dredged materials exhibit little or no toxicity with minimal or very conservative mixing and initial dilution. It is highly unlikely that the toxicity which occurred with prolonged exposure to high concentrations would be observed in the field. Such conditions occur only inside the dredge pipe or hopper and barge bin. The intermittent nature of discrete dumping operations and the relatively rapid dispersion[838,839,840] of released contaminants makes the likelihood of significant acute toxicity to aquatic organisms extremely remote. A suspended particulate phase bioassay suitable for a large variety of

aquatic organisms is presented in detail in the Implementation Manual for Section 103 of Public Law 92-532.[831] Algal bioassays are not recommended for use in the suspended particulate phase because of interferences and predation on the test species by indigenous protozoans in the dredged material being tested and suspended particulates in the test media. Zooplankton were recommended as a test species for suspended particulate bioassays in place of algae.[831]

207. The impact of aquatic disposal of dredged materials upon benthic and epibenthic organisms may potentially be more pronounced than water-column impacts. These organisms are in close contact with deposited dredged materials for long periods of time in contrast to the short exposure of water column organisms.

208. Benthic bioassays with dredged material have been even more limited than water column bioassays with dredged materials. Gannon and Beeton[841] conducted benthic bioassays and sediment selectivity tests with dredged material. Their results, however, are open to question since oxygen depletion in the overlying water caused by high oxygen demand from some sediments may have caused the death of test organisms, rather than any toxic materials that may have been present in the sediments.

209. Bioassay results suggest that dredged sediments can, under some circumstances, exert adverse effects upon benthic and epibenthic organisms that survive burial during the disposal operation or recolonize the site after disposal operations cease. Benthic bioassays showed some degree of toxicity to freshwater grass shrimp (P. kadiakensis) following 6 days of exposure to Bailey Creek, Virginia, sediments.[834] Benthic bioassays also showed that sediments from Bay Ridge Channel in New York City were toxic to M. bahia (oppossum shrimp); sediments from Perth Amboy Channel in New York City were toxic to grass shrimp larvae; and Vicksburg, Mississippi, sediments subject to sewage and chemical plant contamination were highly toxic to adult grass shrimp.[834]

210. Lee et al.[838] evaluated the accumulation of chlorinated hydrocarbon pesticides and PCB's within aquatic organisms residing in the area of several aquatic disposal sites. Even in sediment containing

very high concentrations of these compounds, none of the organisms in the monitored sites exhibited elevated body burdens. Shuba et al.[834] did note uptake and release of kepone by the Asiatic clam Corbicula fluminea. The work of Shuba et al.[834] in conjunction with similar work by Swartz et al.[842] of the US EPA has led to the development of practical benthic bioassay procedures which have been suggested for use in implementation of dredged material disposal criteria.[831] The benthic bioassay procedures are designed to approximate conditions found within or at disposal site boundaries and can determine whether a biological effect is likely. The benthic bioassay procedure measures the overall chemical impact of the dredged material, and cannot be used to determine the constituent causing any biological effect observed.[831] The benthic bioassay procedure can be used to determine the sensitivity of a wide variety of benthic organisms.[834,842]

211. Benthic bioassay procedures, due to their recent development are relatively basic and much work remains before more sophisticated tests can be developed. At present there is no quantitative method for estimating the meaning of a difference between exposed and control test animals and how that difference might be assumed to predict the occurrence of ecologically adverse impacts in the field. Before this need can be addressed, additional study is needed to determine the consistency and variability of current and projected benthic bioassay procedures.

212. Relatively simple and implementable liquid, suspended particulate, and solid phase bioassays have been developed for assessing the impact of dredging and disposal operations upon aquatic organisms. The bioassay results must be interpreted in relation to the dilution that occurs under field conditions. Detailed bioassay interpretation procedures are given in the appropriate dredged material disposal criteria implementation manuals.[831,836]

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Table 1

Commonly Used Species, Reported in Bioassay Literature

Species	Habitat	Reference Numbers
PLANTS		
Chlamydomonas sp. (green)	Marine, planktonic	458, 549, 583, 597, 605, 650, 721
Chlorella pyrenoidosa (green)	Freshwater, planktonic	480, 584, 589, 735, 789, 791, 820, 821
Chlorella sp. (green)	Marine, planktonic	549, 572, 605, 650
Chlorococcum sp. (green)	Marine, planktonic	605, 637, 638, 650
Cyclotella nana (diatom)	Marine, planktonic	542, 556, 573, 575, 576, 605, 647
Dunaliella tertiolecta (green)	Marine, planktonic	556, 576, 577, 605, 637, 638, 668, 782
Euglena gracilis (green)	Freshwater, planktonic	539, 643, 792, 812
Isochrysis galbana (diatom)	Estuarine, planktonic	575, 576, 605, 637, 638, 647, 650
Monochrysis lutheri (diatom)	Marine, planktonic	542, 572, 605, 634, 637, 650
Nitzchia closterium (Phaeodactylum tricornutum) (diatom)	Marine, planktonic	285, 458, 542, 549, 556, 572, 589, 597, 605, 638, 650, 735
Skeletonema costatum (diatom)	Marine, planktonic	285, 542, 549, 556, 573, 575, 576, 583, 626, 647
MOLLUSCS		
Crassostrea gigas (oyster)	Marine, estuarine	301, 485, 535, 558, 562, 629, 677, 686, 752, 811
C. virginica (oyster)	Marine, estuarine	442, 470, 487, 521, 564, 604, 630, 632, 636, 649, 657, 680, 682, 686
Mercenaria mercenaria (quahog)	Marine	286, 442, 471, 487, 516, 521, 582, 604, 629, 636, 680, 682, 686
Mya arenaria (soft-shell clam)	Marine, estuarine	381, 470, 590, 591, 666, 686, 717
Mytilus edulis (mussel)	Marine, estuarine	286, 301, 381, 414, 512, 515, 535, 536, 547, 552, 555, 560, 578, 579, 586, 590, 591, 667, 668, 670, 672, 673, 715, 727, 728, 736
Physa heterostropha (snail)	Freshwater	386, 438, 616, 697
ANNELIDS		
Limnodrilus hoffmeisteri (tubificid)	Freshwater	551, 566, 616, 766
ARTHROPODS (Crustaceans)		
Artemia salina (brine shrimp)	Estuarine	492, 581, 651, 695, 718, 769
Balanus balanoides (barnacle)	Marine	512, 515, 536, 601, 682, 729, 750
Callinectes sapidus (blue crab)	Marine, estuarine	468, 470, 612, 657, 678, 714, 723, 724
Crangon septemspinosa (sand shrimp)	Marine, estuarine	381, 512, 725, 762, 763
Daphnia magna (waterflea)	Freshwater	350, 440, 463, 500, 501, 567, 698, 738, 745
D. pulex (waterflea)	Freshwater	297, 353, 524, 738
Gammarus pseudolimnaeous (amphipod)	Freshwater	348, 359, 379, 432
Homarus americanus (lobster)	Marine	286, 341, 482, 483, 559, 676
Palaemonetes kadiakensis (shrimp)	Freshwater	343, 463, 567, 814
P. pugio (grass shrimp)	Marine, estuarine	534, 594, 606, 612, 714
P. vulgaris (grass shrimp)	Marine, estuarine	286, 333, 381, 612
Penaeus aztecus (brown shrimp)	Marine	570, 612, 628
P. duorarum (pink shrimp)	Marine	557, 570, 612, 628
ARTHROPODS (Insects)		
Acroneuria lycorias (stonefly)	Freshwater	486, 706, 707
A. pacifica (stonefly)	Freshwater	327, 384, 385, 396, 488
Ephemerella subvaria (mayfly)	Freshwater	486, 706, 707
Hydropsyche betteni (caddisfly)	Freshwater	486, 706, 707
Pteronarcys californica (stonefly)	Freshwater	327, 384, 385, 396, 488, 800
FISHES		
Carassius auratus (goldfish)	Freshwater	303, 304, 321, 328, 335, 342, 353, 354, 358, 363, 407, 411, 443, 448, 449, 452, 453, 495, 497, 502, 503, 517, 529, 585, 644, 652, 705, 748, 779, 788, 830,
Catostomus commersoni (white sucker)	Freshwater	280, 295, 342, 349, 352, 380, 495, 530, 705, 753, 803
Cyprinodon variegatus (sheepshead minnow)	Estuarine	305, 311, 314, 368, 381, 458, 540, 570, 595, 819
Cyprinus carpio (carp)	Freshwater	321, 326, 334, 342, 361, 375, 380, 495, 525, 645, 705, 744
Esox lucius (Northern pike)	Freshwater	323, 342, 346, 349, 353, 705, 733, 753
Fundulus heteroclitus (mummichog)	Estuarine, marine	286, 291, 315, 330, 381, 455, 475, 491, 714, 819
Gambusia affinis (mosquitofish)	Freshwater, estuarine	311, 325, 339, 351, 361, 363, 392, 459, 522, 527, 603, 711, 746, 776, 798, 802, 804

(Continued)

Table 1 (Concluded)

Species	Habitat	Reference Numbers
FISHES (Continued)		
Gasterosteus aculeatus (threespine stickleback)	Estuarine, freshwater	301, 351, 356, 399, 465, 730, 777, 781
Ictalurus melas (black catfish)	Freshwater	304, 321, 334, 342, 380, 392, 705
I. natalis (yellow bullhead)	Freshwater	296, 352, 734, 746, 751
I. punctatus (channel catfish)	Freshwater	300, 304, 321, 324, 342, 392, 398, 538, 571, 624, 700, 705, 710, 725, 726
Lagodon rhomboides (pinfish)	Marine	499, 570, 612, 618
Leiostomus xanthurus (spot)	Marine	311, 355, 464, 507, 618
Lepomis cyanellus (green sunfish)	Freshwater	299, 304, 324, 334, 342, 354, 380, 392, 444, 448, 453, 620, 692, 705, 746
L. gibbosus (pumpkinseed)	Freshwater	295, 436, 525, 645, 794
L. macrochirus (bluegill)	Freshwater	281, 288, 299, 301, 309, 312, 313, 320, 321, 324, 328, 334, 336, 342, 349, 351, 352, 353, 354, 357, 362, 365, 366, 369, 370, 386, 388, 389, 392, 398, 408, 411, 433, 434, 435, 437, 438, 440, 443, 448, 451, 452, 457, 481, 489, 490, 497, 503, 509, 517, 518, 529, 543, 615, 631, 683, 684, 693, 697, 703, 705, 716, 726, 734, 737, 759, 760, 761, 779, 794, 796, 806, 818, 819
Micropterus dolomieui (smallmouth bass)	Freshwater	299, 352, 503, 683, 684, 705
M. salmoides (largemouth bass)	Freshwater	295, 321, 324, 334, 352, 354, 357, 358, 369, 375, 380, 392, 452, 494, 503, 631, 653, 683, 684, 703, 704, 705, 725, 734, 779, 794, 796, 802, 826
Morone saxatilis (striped bass)	Freshwater, marine	286, 412, 525, 548, 708, 713, 775
Mugil cephalus (mullet)	Marine	311, 315, 330, 455, 532
Notemigonus crysoleucas (golden shiner)	Freshwater	324, 334, 352, 358, 367, 392, 452, 508, 517, 529, 538, 613, 684, 746, 779, 830
Oncorhynchus kisutch (silver or coho salmon)	Freshwater, marine	316, 321, 351, 372, 387, 391, 399, 421, 426, 441, 485, 610, 655, 656, 663, 675, 685, 703, 704, 705, 773
O. nerka (sockeye salmon)	Freshwater, marine	382, 439, 663, 771, 773, 786
O. tschawytscha (chinook salmon)	Freshwater, marine	351, 372, 391, 461, 548, 656, 669, 703, 704, 774
Perca flavescens (yellow perch)	Freshwater	295, 321, 352, 380, 705, 768, 807
Pimephales promelas (fathead minnow)	Freshwater	298, 317, 319, 321, 331, 349, 352, 354, 357, 358, 376, 379, 401, 403, 411, 419, 420, 443, 448, 451, 453, 484, 489, 497, 502, 541, 543, 574, 593, 623, 696, 705, 731, 779, 787, 801, 807
Poecilia reticulata (guppy)	Freshwater	292, 293, 445, 524, 798, 815, 830
Salmo gairdneri (rainbow trout)	Freshwater	280, 295, 304, 306, 318, 321, 326, 337, 342, 349, 351, 352, 353, 363, 372, 373, 377, 380, 387, 389, 392, 395, 398, 402, 404, 411, 421, 422, 423, 424, 425, 427, 428, 431, 432, 467, 476, 477, 478, 479, 483, 496, 504, 528, 530, 646, 655, 656, 658, 669, 684, 705, 733, 753, 755, 771, 778, 818, 829
S. salar (Atlantic salmon)	Freshwater, marine	289, 341, 374, 378, 397, 400, 406, 482, 483, 520, 523, 533, 554, 601, 602, 609, 661, 749, 817
Salvelinus fontinalis (brook trout)	Freshwater, marine	282, 329, 338, 345, 347, 371, 372, 400, 409, 411, 430, 462, 495, 608, 655, 684, 705, 749, 758
Stizostedion v. vitreum (walleye)	Freshwater	280, 301, 310, 332, 349, 352, 353, 359, 411, 415, 530, 622, 705, 753, 757

Table 2

Infrequently Used Species

Species	Reported Bioassay Habitat	Reference Numbers
BACTERIA/YEASTS/FUNGI		
Arizona arizonae (bacteria)		598
Bacteria (unidentified)		447, 454
Enterobacter aerogenes (bacteria)		598
Escherichia coli (bacteria)		450, 598, 825
Geotrichum candidum (bacteria)		596
Proteus rettgeri (bacteria)		598
Rhodotorula rubra (yeast)		640
Salmonella senftenberg (bacteria)		598
Shigella flexneri (bacteria)		598
Staphylococcus aureus (bacteria)		460
Vibrio marinus (bacteria)		797
PLANTS (Algae)		
Achnanthes brevipes (diatom)	Marine, planktonic	605
Actinocyclus sp. (algae)	Marine, planktonic	650
Amphidinium carteri (diatom)	Marine, planktonic	575, 576, 647
Amphiprora sp. (algae)	Marine, planktonic	650
Amphora exigna (diatom)	Marine, planktonic	605
Amphora sp. (diatom)	Marine, planktonic	650
Anabaena flos-aquae (bluegreen)	Freshwater, planktonic	785
Asterionella japonica (diatom)	Marine, planktonic	626
Blepharisma intermedium (algae)	Freshwater, planktonic	747
Chaetoceros compressus (diatom)	Marine, planktonic	626
C. debilis (diatom)	Marine, planktonic	626
C. decipiens (diatom)	Marine, planktonic	285
C. pelagicus (diatom)	Marine, planktonic	668
Chlamydomonas moewusii (green)	Freshwater, planktonic	738
C. reinhardtii (green)	Freshwater, planktonic	524, 544
Chlorella vannielli (green)	Freshwater, planktonic	473
C. vulgaris (green)	Freshwater, planktonic	413, 545
Chlorococcum macrostrigmatum (green)	Freshwater, planktonic	493
Cladophora sp.	Marine, freshwater, benthic	460, 532
Coccochloris elabans (bluegreen)	Marine, planktonic	556
Cyclotella sp.	Freshwater, planktonic	721
Cylindrotheca closterium (diatom)	Marine, planktonic	626
Dicrateria inornata (green)	Marine, planktonic	637
Dunaliella euchlora (green)	Marine, planktonic	542, 572, 650
Dunaliella sp. (green)	Marine, planktonic	579
Eucampa zodiacus (algae)	Marine, planktonic	626
Euglena viridas (green)	Freshwater, planktonic	413
Exuviella sp. (red)	Marine, planktonic	556, 668
Fontinalis antipyretica (green)	Freshwater, planktonic	790
Fragilaria sp. (diatom)	Freshwater, planktonic	721
Glenodinium foliaceum (red)	Marine, planktonic	556
Glenodinium sp. (red)	Marine, planktonic	556
Guinordia flaccida (algae)	Marine, planktonic	626
Gyrodinium resplendens (algae)	Marine, planktonic	668
Laminaria saccharina (brown)	Marine, benthic	561, 664, 742
Leptocylindricus minimus (diatom)	Marine, planktonic	626
Leptocylindricus sp. (diatom)	Marine, planktonic	573
Licmorpha lyngbyei (diatom)	Marine, planktonic	285, 626
Macrocystis pyrifera (brown)	Marine, benthic	599
Mishococcus sp. (diatom)	Marine, planktonic	458
Nannochloris sp. (diatom)	Marine, planktonic	637
Navicula inserta (diatom)	Marine, planktonic	605
Neochloris sp. (green)	Marine, planktonic	605
Nitzschia laevis (diatom)	Marine, planktonic	650
N. linearis (diatom)	Freshwater, planktonic	386
N. seriata (diatom)	Marine, planktonic	626
Nitzschia sp. (diatom)	Marine, planktonic	536
Olisthodiscus luteus (algae)	Marine, planktonic	576
Paralia sulcata (algae)	Marine, planktonic	626
Pediastrum tetras (green)	Freshwater, planktonic	413
Periphyton (unidentified)	Freshwater, benthic	418, 824
Platymonas suecia (green)	Marine, planktonic	647
Platymonas sp. (green)	Marine, planktonic	458, 605, 650
Pleurosigma angulatum (algae)	Marine, planktonic	626
Porphyridium cruentum (red)	Marine, planktonic	458, 605
Protococcus sp. (green)	Marine, planktonic	572, 650
Rhizosolenia fragilissima (diatom)	Marine, planktonic	626
Scenedesmus quadricaudata (green)	Freshwater, planktonic	641
Scenedesmus sp. (green)	Freshwater, planktonic	721

(Continued)

Table 2 (Continued)

Species	Reported Bioassay Habitat	Reference Numbers
PLANTS (Algae) (Continued)		
Selenastrum capricornutum (green)	Freshwater, marine, planktonic	513, 537, 635
S. gracile (green)	Freshwater, planktonic	799
Stauroneis amphoroides (diatom)	Marine, planktonic	605
Synedra sp. (diatom)	Freshwater, planktonic	721
Tetrahymena pyriformis (ciliate)	Freshwater, planktonic	514
T. vorax (ciliate)	Freshwater, planktonic	524
Thalassionema nitzchoides (algae)	Marine, planktonic	626
Thalassiosira fluviatilis (diatom)	Marine, planktonic	556, 605
T. levanderi (diatom)	Marine, planktonic	626
T. nordenskioldii (diatom)	Marine, planktonic	626
Vaucheria sp.	Freshwater, benthic	460
PLANTS (Flowering Plants)		
Alternanthera philoxeroides (alligator-weed)	Freshwater	793
Azolla caroliniana (azolla)	Freshwater	795
Ceratophyllum dermersum (coontail)	Freshwater	794
Chara vulgaris (muskgrass)	Freshwater	794
Distichlis spicata (marsh grass)	Estuarine	550
Lemna minor (duckweed)	Estuarine	429, 793, 795
Najas flexilis (pondweed)	Estuarine	794
Potamogeton diversifolius (pondweed)	Estuarine	794
P. foliosus (pondweed)	Estuarine	794
P. pectinatus (pondweed)	Estuarine	794
P. pusillus (pondweed)	Estuarine	794
Rhizophora mangle (mangrove)	Marine	639
Spartina alterniflora (marsh grass)	Estuarine	550, 611
S. patens (marsh grass)	Estuarine	550
Spirodela polyrhiza (giant duckweed)	Estuarine	795
Wolffia columbiana (watermeal)	Estuarine	795
Wolffiella floridana	Estuarine	795
PROTOZOANS		
Coccolithus hyxleyi (coccolith)	Marine	647, 772, 823
COELENTERATES		
Bimeria franciscana (hydroid)	Marine	722
Chrysaora quinquecirrha (medusa)	Marine	741
Hydra sp. (hydroid)	Freshwater	302
Phialidium gregarium (medusa)	Marine	662
Sarsia tubulosa (medusa)	Marine	662
CTENOPHORES		
Pleurobrachia pileus (comb jelly)	Marine	662
BRYOZOANS		
Bugula neritina (erect)	Marine	769
Watersipora cucullata (encrusting)	Marine	769
PLATHELMINTHES		
Dugesia sp.	Freshwater	440
Turbellarian (unidentified)	Freshwater	302
ECHINODERMS		
Asterias forbesi (starfish)	Marine	286, 381
Crossaster papposus (starfish)	Marine	562
Dendraster excutricus (starfish)	Marine	562
Luidia foliata (starfish)	Marine	562
Pisaster ohraceous (starfish)	Marine	562
Strongylocentrotus purpuratus (urchin)	Marine	767
MOLLUSKS		
Aequipecten irradians (scallop)	Marine	680
Amnicola sp.	Freshwater	816
Arctica islandica (clam)	Marine	512
Campeloma decisum (snail)	Freshwater	348, 432
Clinocardium nuttalli (cockle)	Marine	301, 344
Gemma gemma (clam)	Marine	666
Goniobasis sp. (snail)	Freshwater	364
Haminoea virescens (limpet)	Marine	562
Katharina tunicata (chiton)	Marine	562
Lymnaea sp. (snail)	Freshwater	440
Macoma balthica (clam)	Marine, estuarine	390, 666, 717
M. phenax (clam)	Marine, estuarine	390
Melibe lemina (limpet)	Marine	562
Modiolus demissus (mussel)	Estuarine	547

(Continued)

Table 2 (Continued)

Species	Reported Bioassay Habitat	Reference Numbers
MOLLUSKS (Continued)		
Mulinia lateralis (clam)	Marine	666, 717
Mytilus californianus (mussel)	Marine	667, 668
Nassa obsoleta (snail)	Marine	333
Nassarius obsoletus (snail)	Marine	286, 381
Physa integra (snail)	Freshwater	348, 432
Physa sp. (snail)	Freshwater	302
Rangia cuneata (clam)	Marine, estuarine	390, 813
Spisula solidissima (clam)	Marine	512
Unionidae (clam)	Freshwater	302
Urosalpinx cinerea (oyster drill)	Estuarine	381
ANNELIDS		
Capitella capitata (polychaete)	Marine	417, 553, 580
Clymenella torquata (polychaete)	Marine	671
Diopatra cuprea (polychaete)	Marine	671
Dorvillea articulata (polychaete)	Marine	417
Leeches	Freshwater	302
Limnodrilus udekemianus (tubificid)	Freshwater	766
Nais sp. (oligochaete)	Freshwater	816
Neanthes arenaceodentata (polychaete)	Marine	417
Nereis branti (sandworm)	Marine, estuarine	562
N. grubbei (sandworm)	Marine, estuarine	417
N. diversicolor (sandworm)	Marine	283, 780, 783, 784
N. vexillosa (sandworm)	Marine	562
N. virens (sandworm)	Marine	286, 592
Polydora sp. (polychaete)	Marine	594
Serpula vermicularis (tubeworm)	Marine	562
Tubifex tubifex (tubificid)	Freshwater	551, 566
ARTHROPODS (Crustaceans)		
Acartia clausii (copepod)	Estuarine, marine	651
A. tonsa (copepod)	Estuarine, marine	390, 534, 594
Asellus brevicaudus (isopod)	Freshwater	567
A. militaris (isopod)	Freshwater	416, 690
Asellus sp. (isopod)	Freshwater	302
Balanus cariosus (barnacle)	Marine	562
B. eburneus (barnacle)	Estuarine, marine	472
B. improvisus (barnacle)	Estuarine	534
Calanus finmarchicus (copepod)	Marine	633
Callianasa californianus (ghost shrimp)	Marine	301
Cancer irroratus (crab)	Marine	512
C. magister (crab)	Marine	301, 340, 660
Carcinus maenus (crab)	Marine	381, 536, 560, 563, 590, 674, 732
Chlamydotheca arcuata (ostracod)	Freshwater	287, 654
Cyclops varicans (copepod)	Freshwater	822
Cypretta kawatai (ostracod)	Freshwater	619
Cypridopsis vidua (ostracod)	Freshwater	524, 567
Daphnia melanogaster (cladoceran)	Freshwater	745
Eubranchipus moorei (crustacean)	Freshwater	690
Euchaeta japonica (copepod)	Marine	410, 565
Eulimnadia inflecta (conchostracan)	Freshwater	690
Euphasusia pacifica (euphausiid)	Marine	699
Eurytemora affinis (copepod)	Estuarine, Marine	390
Gambarus sp. (crayfish)	Freshwater	302
Gammarus fasciatus (amphipod)	Freshwater	416, 567
G. figrinus (amphipod)	Estuarine	534, 581
G. gammarus (amphipod)	Freshwater	463
G. lacustris (amphipod)	Freshwater	323
G. oceanicus (amphipod)	Marine	289, 341, 454
Gammarus sp. (amphipod)	Freshwater	302, 816
Hemigrapsus oregonensis (crab)	Marine	301
Hepatus epheliticus (crab)	Estuarine, marine	678
Hyalella azeteca (amphipod)	Freshwater	383, 814
Leptodius floridanus (crab)	Marine	568, 569
Limnoria lignorum (gribble)	Marine, estuarine	617
L. quadripunctata (gribble)	Marine, estuarine	617
L. tripunctata (gribble)	Marine, estuarine	617
Melita nitida (amphipod)	Estuarine	534
Orconectes nais (crayfish)	Freshwater	394, 463, 567
O. rusticus (crayfish)	Freshwater	364
Pagurus longicarpus (hermit crab)	Marine	286, 381
P. pollicaris (hermit crab)	Marine	512
Paleomonetes sp. (grass shrimp)	Estuarine	458

(Continued)

Table 2 (Continued)

Species	Reported Bioassay Habitat	Reference Numbers
ARTHROPODS (Crustaceans) (Continued)		
Panopeus herbstii (crab)	Marine	568, 678
Pontoporeia affinis (amphipod)	Freshwater	279, 405
Procamburus acutus (crayfish)	Freshwater	526
P. blandingi (crayfish)	Freshwater	642
P. clarki	Freshwater	360, 709, 712
P. simulans (crayfish)	Freshwater	394
Pseudocalanus sp. (copepod)	Marine	633
Pseudodiaptomus cornutus (copepod)	Marine	285
Pseudodiaptomus sp. (copepod)	Marine	594
Rhithropanopeus harrisii (crab)	Estuarine, marine	678, 688
Scottolana canadensis (copepod)	Estuarine	390
Sesarma cinereum (crab)	Estuarine, marine	678, 764
Simocephalus serratulatus (cladoceran)	Freshwater	297
Streptocephalus seali (anostracan)	Freshwater	690
Temora longicornis (copepod)	Marine	633
Tigriopus californianus (copepod)	Marine	519
Tortanus discaudatus (copepod)	Marine	827
Uca minax (fiddler crab)	Estuarine	311
Uca pugilator (fiddler crab)	Marine, estuarine	612, 614, 764
Uca pugnax (fiddler crab)	Estuarine	764
Upogebia pugettensis (mud shrimp)	Marine	301
ARTHROPODS (Insects)		
Agrion sp. (damselfly)	Freshwater	740
Anax sp. (dragonfly)	Freshwater	740
Anopheles quadrimaculatus (mosquito)	Freshwater	498, 508
Arctopsyche grandis (caddisfly)	Freshwater	327, 488
Atherix sp. (snipefly)	Freshwater	302
Baetisca laurentina (mayfly)	Freshwater	706
Boyeria vinuosa (dragonfly)	Freshwater	707
Boyeria sp. (dragonfly)	Freshwater	302
Brachycentrus americanus (caddisfly)	Freshwater	707
Callibaetis sp. (mayfly)	Freshwater	383
Cheumatopsyche sp. (caddisfly)	Freshwater	302, 364
Chironomus plumosus (midge)	Freshwater	765
C. staegeri (midge)	Freshwater	765
C. tentans (midge)	Freshwater	805
Chironomus sp. (midge)	Freshwater	463, 816
Chloroperia sp. (stonefly)	Freshwater	302
Claasenia sabulosa (stonefly)	Freshwater	327, 488, 800
Corixidae (water boatman)	Freshwater	302
Corydalus cornutus (hellgrammite)	Freshwater	613
Culex pipiens (mosquito)	Freshwater	463
Culex sp. (mosquito)	Freshwater	440
Dineutes americanus (beetle)	Freshwater	814
Enallagma sp. (damselfly)	Freshwater	383
Ephemera simulans (mayfly)	Freshwater	486, 706, 707
Ephemerella sp. (mayfly)	Freshwater	302
Gomphus sp. (dragonfly)	Freshwater	302
Hexagenia bilineata (mayfly)	Freshwater	463
H. limbata (mayfly)	Freshwater	706
Hexagenia sp. (mayfly)	Freshwater	302, 308
Hydroperla crosbyi (stonefly)	Freshwater	613
Hydropsyche californica (caddisfly)	Freshwater	327
Ischnura verticalis (mayfly)	Freshwater	463
Isogenus tontinalis (stonefly)	Freshwater	707
Isonychia bicolor (mayfly)	Freshwater	364
Isonychia sp. (mayfly)	Freshwater	302
Isoperla sp. (stonefly)	Freshwater	302
Leptophlebia nebulosa (mayfly)	Freshwater	706
Lestes eurinus (damselfly)	Freshwater	691
Lestes sp. (damselfly)	Freshwater	690
Lethocercus sp. (giant waterbug)	Freshwater	302
Libellula sp. (dragonfly)	Freshwater	383, 463
Limnephilus sp. (caddisfly)	Freshwater	383
Macromia sp. (dragonfly)	Freshwater	814
Ophiogomphus rupinsulensis (dragonfly)	Freshwater	707
Ophiogompha sp. (dragonfly)	Freshwater	302
Paragnetina sp. (stonefly)	Freshwater	302
Phrygnea sp. (caddisfly)	Freshwater	740
Procladius sp. (insect)	Freshwater	616
Pteronarcella badia (stonefly)	Freshwater	800
Pteronarcys dorsata (stonefly)	Freshwater	706, 707

(Continued)

Table 2 (Continued)

Species	Reported Bioassay Habitat	Reference Numbers
ARTHROPODS (Insects) (Continued)		
Simulidae (blackfly)	Freshwater	302
Simulium tuberosum (blackfly)	Freshwater	754
S. venustum (blackfly)	Freshwater	754
S. verecandum (blackfly)	Freshwater	754
Siphlonurus sp. (mayfly)	Freshwater	463, 740
Stenonema ares (mayfly)	Freshwater	364
S. heterotarsale (mayfly)	Freshwater	364
Stenonema rubrum (mayfly)	Freshwater	707
Stenonema sp. (mayfly)	Freshwater	302
Stictochironomus annulierus (bloodworm)	Freshwater	616
Taeniopteryx maura (stonefly)	Freshwater	707
Tanytarsus dissimilis (midge)	Freshwater	621, 706
Tanytarsus sp. (midge)	Freshwater	765
Tendipedidae sp. (bloodworm)	Freshwater	302, 383
Tetragoneuria cynosura (dragonfly)	Freshwater	625
T. semiagua (dragonfly)	Freshwater	625
Triaenoides sp. (caddisfly)	Freshwater	302
Tropiternus lateralis (beetle)	Freshwater	756
FISHES		
Adinia xenica (killifish)	Estuarine	311
Agosia chrysogaster (longfin dace)	Freshwater	763
Alosa aestivalis (blueback herring)	Freshwater	810
A. pseudoharengus (alewife)	Marine, freshwater	648
A. sapidissima (American shad)	Freshwater	469, 665, 694, 808
Ameirus melas (black bullhead)	Freshwater	692
A. nebulosus (brown bullhead)	Freshwater	466, 503, 684
Anguilla rostrata (American eel)	Freshwater, marine	315, 455, 525, 645
Apeltes quadracus (stickleback)	Marine	819
Campostoma anomalum (stoneroller)	Freshwater	299
Carassius carassius (goldfish)	Freshwater	440
Catastomus clarki (gila sucker)	Freshwater	689
Chasmodes bosquianus (striped blenny)	Estuarine	719
Clupea harengus (herring)	Marine	341, 587, 588, 627
Cottus bairdii (sculpin)	Freshwater	295
C. perplexus (sculpin)	Freshwater	685
Ctenopharyngodon idellus (grass carp)	Freshwater	705
Cymatogaster aggregata (shiner perch)	Estuarine, marine	290, 301, 607
Cyprinodon macularius (desert pupfish)	Freshwater	679, 689
Engraulis mordax (northern anchovy)	Marine	505
Erimyzon sucetta (lake chubsucker)	Freshwater	299
Etheostoma lepidum (greenthroat darter)	Freshwater	739
Eucalia inconstans (stickleback)	Freshwater	743
Fundulus diaphanus (banded killifish)	Freshwater	525, 645, 705
F. grandis (killifish)	Estuarine	311
F. majalis (killifish)	Estuarine	315, 333, 381
F. similis (killifish)	Estuarine	311, 458
Fundulus sp. (killifish)	Estuarine	311
Gadus morhua (Atlantic cod)	Marine	397, 515, 579, 587, 590
Girella nigricans (greenfish)	Marine	770
Gobiesox strumosus (skilletfish)	Estuarine	719
Gobionellus boleosoma (goby)	Estuarine	311
Gobiosoma bosci (naked goby)	Estuarine	719
Harengula pensacolae (sardine)	Marine	809
Ictalarus nebulosus (brown bullhead)	Freshwater	295, 796, 818, 828
Ictiobus cyprinellus (bigmouth buffalo)	Freshwater	324
Jordanella floridae (flagfish)	Freshwater	456, 610
Kuhlia sandvichensis (aholehole)	Marine	798
Lepomis humilis (orangespotted sunfish)	Freshwater	692
L. microlophus (redear sunfish)	Freshwater	321, 681, 794, 796
Lucania parva (killifish)	Estuarine	311
Megastomatobus cyprinella (buffalo)	Freshwater	779
Menidia beryllina (silverside)	Estuarine	311
M. menidia	Estuarine, marine	315, 330, 455, 694
Micrometrus minimus (dwarf shiner)	Marine, estuarine	339, 607
Micropogon undulatus (croaker)	Marine, estuarine	507, 570, 657
Moxostoma aureoleum (redhose)	Freshwater	692
Notropis a. atherinoides (minnow)	Freshwater	702
N. blennis (minnow)	Freshwater	692
N. cornutus (minnow)	Freshwater	295
N. heterolepis (bluntnose shiner)	Freshwater	352
N. rubellus (minnow)	Freshwater	702

(Continued)

Table 2 (Concluded)

Species	Reported Bioassay Habitat	Reference Numbers
FISHES (Continued)		
N. spilopterus (minnow)	Freshwater	702, 725
Oligocottus snyderi (fluffy sculpin)	Marine	393
Oncorhynchus gorbuscha (pink salmon)	Freshwater, marine	546, 663
Opsanus tau (toadfish)	Marine	657, 682
Osmerus mordax (smelt)	Marine	347
Parophrys vetulus (English sole)	Marine	301
Percina caprodes (logperch)	Freshwater	295
Petromyzon marinus (sea lamprey)	Freshwater	295, 352
Pimephales notatus (bluntnose minnow)	Freshwater	510, 692, 702, 796
Poecilia latipinna (silfin molly)	Freshwater, estuarine	305, 311, 322, 440
Pomatomus saltatrix (bluefish)	Marine	315
Pomoxis annularis (white crappie)	Freshwater	392, 503
P. nigromaculatus (black crappie)	Freshwater	683, 684, 779
Pseudopleuronectes americanus (flounder)	Marine	315, 455, 694
Ptychocheilus oregonensis (squawfish)	Freshwater	372
P. umpquae (squawfish)	Freshwater	372
Rhinichthys a. atratulus (bluntnose dace)	Freshwater	701
R. osculus (speckled dace)	Freshwater	689
Salmo clarki (cutthroat trout)	Freshwater	511, 600, 655
S. trutta (brown trout)	Freshwater	306, 321, 684, 705
Salvelinus namaycush (lake trout)	Freshwater	705
Sebastodes miniatus (Pacific rockfish)	Marine	705, 720
Semotilis a. atromaculatus (creek chub)	Freshwater	701
Sphaeroides maculatus (northern puffer)	Marine	315, 694
Stenotomus chrysops (scup)	Marine	286
Stolephorus purpurea (nehu)	Marine	798
Syngnathus fuscus (northern pipefish)	Marine	315
Tautogolabrus adspersus (cunner)	Marine, estuarine	512, 819
Tilapia mossambica (tilapia)	Freshwater	798
Tilapia sp. (tilapia)	Freshwater	334
Trinectes maculatus (hogchoker)	Marine, estuarine	390, 474, 687
Xiphophorus maculatus (platyfish)	Freshwater	403
AMPHIBIANS		
Rana catesbeiana (bullfrog)	Freshwater	324, 334
Rana pipiens (leopard frog)	Freshwater	334
Rana sp. (frog)	Freshwater	440
BIRDS		
Anas platyrhynchos (mallard duck)	Freshwater	284

Table 3

Test Species Previously Used in Turbidity and Turbidity-Related Bioassays

Species	Life Cycle Stage	Type of Turbidity	Reference Numbers
Acartia tonsa	Adults	Sludge	594
Bacteria			
Escherichia coli	Not applicable	Stream sediment eluates	598
Enterobacter aerogenes	Not applicable	Stream sediment eluates	598
Proteus rettgeri	Not applicable	Stream sediment eluates	598
Arizona arizonae	Not applicable	Stream sediment eluates	598
Shigella flexneri	Not applicable	Stream sediment eluates	598
Salmonella senftenberg	Not applicable	Stream sediment eluates	598
Balanus balanoides	Adults	Oil and carbonized sand	682
Callinectes sapidus	Adults	Sediment-sorbed radioactive gold	657
Catostomus commersoni	Adult	Paper mill effluent	295
Chaetoceros decipiens	Adults	Acid-iron wastes	285
C. pelagicus	Adults	Chromium and lead in marine sewage outfall sediments	668
Cottus bairdii	Adult	Paper mill effluent	295
Crassostrea gigas	Larvae/adults	Pulp mill wastes	677
	Adults	Suspended silt, kaolin, fuller's earth	811
C. virginica	Adults	Turbidity, siltation from dredging operations	294
	Larvae	Turbidity-producing substances	604
	Adults/larvae	Silt and kaolin turbidity	636
		Suspended silt	649
	Adults	Oil and carbonized sand	682
Cyprinodon variegatus	Adult	Various types of silt	819
Dunaliella tertiolecta	Adults	Chromium and lead in marine sewage outfall sediments	668
Euchaeta japonica	Eggs/larvae	Copper and marine sediment extracts	410
	Adults	Affect of particulate clay minerals, diatoms, ascorbic acid, humic acid, sewage effluent and soil extracts	565
Exuviella sp.	Adults	Chromium and lead in marine sewage outfall sediments	668
Fundulus heteroclitus	Adult	Various types of silt	819
Gammarus pseudolimnaeous		Paper fiber sludge	359
Gasterosteus aculeatus	Adults	Harbor sediment contaminants	399
Gyrodinium resplendens	Adults	Chromium and lead in marine sewage outfall sediments	668
Homarus americanus	Larvae/adults	Kraft pulp mill effluent	482, 676
Hyalella azeteca	Adult	Herbicides and pond mud	383
Ictalurus nebulosus	Adults	Paper mill effluent	295
Laminaria saccharina	Adults	Silt/various pullutants	742
Lebistes reticulatus	Adults	Paper factory effluents	531
	Adults	Kraft pulp mill effluents	786
Leiostomus xanthurus	Adults	Sediment-sorbed radioactive gold	657
Lepomis cyanellus	Juveniles	Silt, turbidity	620
L. gibbosus	Adults	Paper mill effluent	295
Licmorpha lyngbyei	Adults	Acid-iron wastes	285
Limnodrilus hoffmeisteri	Adults	Detritus-sorbed radioisotopes, transfer to *macroinvertebrates*	616
Limnephilus sp.	Larvae	Herbicides and pond mud	383
Mercenaria mercenaria	Eggs/larvae	Suspended silt	516
	Larvae	Turbidity-producing substances	604
	Adults/larvae	Silt and kaolin turbidity	636
	Adults	Oil and carbonized sand	682
Microflora	Not applicable	Oil dispersant in beach sand	659
Micropterus salmoides	Adults	Paper mill effluent	295
	Juveniles	Suspended silt	620
Mytilus edulis	Embryos	Kraft pulp mill effluent	414
	Adults	Ferric hydroxide turbidity	586, 736
Nereis diversicolor	Adults	Manganese in estuarine sediments	783
	Adults	Zinc and cadmium in estuarine sediments	784
Nitzschia closterium	Adults	Acid-iron wastes	285
Notropis a. atherinoides	Adults	Kraft pulp mill effluent	702
N. cornutus	Adults	Paper mill effluent	295
N. notatus	Adults	Kraft pulp mill effluent	702
N. rebellus	Adults	Kraft pulp mill effluent	702
N. spilopterus	Adults	Kraft pulp mill effluent	702
Oligocottus snyderi	Adults	Kraft pulp mill effluent	393
Oncorhynchus gorbuscha		Kraft pulp mill effluent	663
O. kisutch	Adults/juveniles	Kraft pulp mill effluent	316, 382, 421, 426, 663
O. nerka		Kraft pulp mill effluent	439, 663, 786

(Continued)

Table 3 (Concluded)

Species	Life Cycle Stage	Type of Turbidity	Reference Numbers
Opsanus tau	Adults	Sediment-sorbed radioactive gold	657
	Embryos	Oil and carbonized sand	682
Ostrea edulis	Larvae	Turbidity-producing substances	604
Paleomonetes pugio	Adults	Sludge	594
Perca flavescens	Adults	Paper mill effluent	295
Percina caprodes	Adults	Paper mill effluent	295
Petromyzon marinus	Adults	Paper mill effluent	295
Physa heterostropha	Adults	Detritus-sorbed radioisotopes, transfer to macroinvertebrates	616
Pimephales promelas	Adults	Wood fibers	317
	Adults	Ferric hydroxide turbidity	401
	Adults	Turbidity and endrin	696
Poecilia reticulata	Adults	Mercury-containing sediments	292, 293
Polydora sp.	Larvae	Sludge	594
Pontoporeia affinis	Adults	Harbor and Great Lakes sediments	279
	Adults	Dredged sediments	405
Pseudodiaptomus coronatus	Adults	Acid-iron wastes	285
Pseudodiaptomus sp.	Adults	Sludge	594
Salmo gairdneri		Paper mill effluent	295
	Eggs/fry	Wood fibers	306
	Juveniles	Kraft pulp mill effluent	421
S. salar		Kraft pulp mill effluent	482, 483
S. trutta	Eggs/fry	Wood fibers	306
Salvelinus fontinalis	Juveniles	Turbidity and neutralized $Fe(OH)_2$	282
Skeletonema costatum	Adults	Acid-iron wastes	285
Stictochironomus annulicrus	Adults	Detritus-sorbed radioisotopes, transfer to macroinvertebrates	616
Stizostedion v. vitreum		Wood fibers	307, 310
	Eggs/fry	Wood fibers	332
	Eggs/fry	Paper fiber sludge	359
	Eggs	Wood fibers/slime bacterium	415
Tautogolabrus adspersus	Adults	Various types of silt	819
Tubularia crocea	Adults	Oil and carbonized sand	682
Viruses	Not applicable	Clay particles	446

Table 4

Test Species Which Have Been Used Successfully in Bioassays but Present Some Special Problems

(A brief explanation of problems encountered and reported follows each accession number)

Species	Reference Numbers	Problems
PLANTS		
Laminaria saccharina (brown algae)	664	Can grow too large for laboratory conditions
MOLLUSKS		
Crassostrea gigas (oyster)	677	Some mortality problems among larvae and spat
Clinocardium nuttali (cockle)	344	Unexplained mortalities
Macoma balthica (clam)	390	Poor experimental animal
M. phenax (clam)	390	Poor experimental animal
Mercenaria mercenaria (quahog)	471	Resistant to methoxychlor and malathion
Mytilus edulis (mussel)	536	Problems with laboratory mortality
	552	100 percent control mortality in summer months, feeding problems
	578	Difficult to determine point of death
	586	10 percent control mortality at 4 months, 20 percent at 5 months
	672	High control and experimental mortalities
ARTHROPODS		
Acartia tonsa (copepod)	534	Sensitive to mechanical handling
Artemia salina (brine shrimp)	492	Poor survival rate during development to adult stage due to crowding
Balanus improvisus (barnacle)	534	Larvae sensitive to mechanical handling
Callinectes sapidus (blue crab)	468	High mortality problem
	470	High mortality rate required confinement to cages
	723	Cannibalism problems during ecdysis
Carcinus maenas (crab)	536	Difficult to keep alive due to reluctance to feed in laboratory
Chlamydotheca arcuata (ostracod)	287	Death point difficult to determine
Gammarus oceanicus (amphipod)	341	Difficult to determine point of death
	506	Some cannibalism problems
Homarus americanus (lobster)	482	Artificial seawater not favorable for long survival of larvae
Hydropsyche sp. (caddisfly)	308	Larvae survival beyond 96 hours poor
Lethocerous sp. (giant waterbug)	302	High control mortality
Penaeus duorarum (pink shrimp)	557	4-26 percent control mortality in 15-53 days
Procamburus acutus (crayfish)	526	Cannibalism problems with more than one individual per container
P. clarki (crayfish)	360	Cannibalism problems with more than one individual per container
	709	High mortality during transporting of young
Scottolana canadensis (copepod)	390	Contamination of culture medium caused high mortality
Tropisternus lateralis (beetle)	756	Larvae require separation due to cannibalism problems
FISH		
Alosa aestivalis (blueback herring)	810	Some mortality due to handling stress
A. pseudoharengus (alewife)	648	High mortality rate upon introduction of seawater, fragile
A. sapidissima (American shad)	665	Mortality problems during handling
	694	Death rate accelerated due to overexcitation when placed in clear plexiglass containers
Carassius auratus (goldfish)	335	Problems with Gyrodactylus skin flukes, requiring repeated methylene blue treatments
Cyprinodon macularius (desert pupfish)	689	Pugnacious behavior requires separation from other species
Cyprinus carpio (carp)	366	Field experiments did not work where histological examinations were required
Gambusia affinis (mosquitofish)	339	Treatments with 2.5 percent noniodized table salt required to control tail rot fungus
Gasterosteus aculeatus (threespine stickleback)	351	High mortality among larvae 4 to 7 days after hatching
Leiostomus xanthurus (spot)	387	High control mortality due to crowding and low seawater flow
Lepomis macrochirus (bluegill)	312	High control mortality
	369	Parasitism problems
	408	Dominance-submissive relations develop among fish held together in small containers and affect results
Megalops atlanticus (tarpon)	305	Difficult to keep alive in laboratory, small number available
Micropterus salmoides (largemouth bass)	369	Parasitism problems
Mugil cephalus (mullet)	352	Fish tend to jump out of holding tanks
Notropis cornutus (shiner)	295	Vulnerable to ordinary retention
Osmerus mordax (smelt)	347	Salinity of 33 o/oo caused some control mortality
Pomoxis annularis (white crappie)	620	Suitable for field experiments, but not suitable for laboratory tests due to inability to adapt to aquarium conditions
Ptychocheilus oregonensis (squawfish)	372	Much more sensitive to piscicide than salmonids
P. umpquae (squawfish)	372	Much more sensitive to piscicide than salmonids
Salmo clarki (cutthroat trout)	511	Approximately 20 percent control mortality after 20 months
S. gairdneri (rainbow trout)	778	Problem with fish jumping out of holding tanks
Salmo salar (Atlantic salmon)	400	When trout are handled, air is lost from the swim bladder and must be replaced at the surface before they are able to swim without undue effort
Salvelinus fontinalis (brook trout)	282	High mortality due to outbreak of Ichthyopthirius fungus
	338	Fish established territories and attacked each other when more than one fish was placed in small (317 liter) container
	347	In marine tests salinity of 33 o/oo caused some control mortality
	400	When trout are handled, air is lost from the swim bladder and must be replaced at the surface before they are able to swim without undue effort
Sphaeroides maculatus (northern puffer)	315	Died within 24 hours in 20 liter jars without aeration

Table 5

Rankings of Fish Species on a Scale of 1-8 as Suitable Bioassay Animals[2,4,5]

	Species in Order of Suitability	Availability	Size	Health	Adaptability to Laboratory Life	Metabolic Rate	Acceptance of Food	Does not Jump from Effluent	Total
1.	Poecilia reticulata	8	8	8	8	8	8	8	56
2.	Gambusia affinis	7	8	8	8	8	8	8	55
3.	Pimephales notatus	4	8	8	8	8	8	8	52
4.	Pimephales promelas	4	8	8	8	8	8	8	52
5.	Pimephales vigilax	4	8	8	8	8	8	8	52
6.	Lepomis macrochirus	5	7	8	7	8	8	8	50
7.	Carassius auratus	4	7	8	7	8	8	8	51
8.	Chromosmus erythrogaster	4	8	8	6	8	8	8	50
9.	Lepomis cyanellus	4	7	8	7	8	8	8	50
10.	Notropis boops	4	8	8	6	8	8	8	50
11.	Notropis lutrensis	4	8	8	7	8	8	7	50
12.	Semotilus atromaculatus	4	8	8	6	8	8	8	50
13.	Tilapia nilotica	4	6	8	8	8	8	8	50
14.	Campostoma anomalum	4	8	6	7	8	8	8	49
15.	Lepomis gulosus	2	6	8	7	8	8	8	49
16.	Etheostoma spectabile	4	8	8	7	8	6	8	49
17.	Fundulus olivaceus	3	8	8	6	8	8	8	49
18.	Labidesthes sicculus	4	8	8	7	8	6	8	49
19.	Notropis zonatus	2	8	8	6	8	7	8	49
20.	Lepomis humilus	2	7	8	7	8	8	8	48
21.	Hybognathus placitus	4	7	7	6	8	7	8	47
22.	Lepomis megalotus	3	6	8	6	8	8	8	47
23.	Notropis cornutus	2	7	8	6	8	8	8	47
24.	Notropis (percobromis) atherinoides	4	8	6	6	7	8	8	47
25.	Notropis stramineus	2	8	7	6	8	8	8	47
26.	Cottus carolinae	2	8	6	6	8	8	8	46
27.	Cyprinus carpio	4	4	8	6	8	8	8	46
28.	Culaea inconstans	4	8	8	8	2	8	8	46
29.	Hybognathus hankinsoni	2	8	6	6	8	8	8	46
30.	Hybopsis amblops	2	8	8	6	8	6	8	46
31.	Ictalurus melas	4	4	8	6	8	8	8	46
32.	Notemigonus crysoleucas	4	8	8	6	8	8	6	46
33.	Notropis dorsalis	2	8	8	4	8	8	8	46
34.	Notropis girardi	3	8	7	6	8	6	8	46
35.	Notropis whipplei	2	8	6	6	8	8	8	46
36.	Ambloplites rupestris	2	6	8	8	8	5	8	45
37.	Lepomis microlophus	4	7	8	6	8	4	8	45
38.	Notropis venustus	2	8	6	6	8	8	7	45
39.	Micropterus salmoides	2	4	8	6	8	8	8	44
40.	Menidia audens	2	8	6	6	8	6	8	44
41.	Notropis camurus	2	8	8	4	8	6	8	44
42.	Lepomis auritus	3	6	7	6	8	6	8	43
43.	Ictalurus nebulosus	2	4	8	4	8	8	8	42
44.	Noturus exilis	2	8	4	6	8	6	8	42
45.	Ictalurus punctatus	2	4	7	4	8	8	8	41
46.	Ictiobus cyprinellus	4	4	6	1	8	8	8	41
47.	Notropis rubellus	1	8	4	4	8	6	8	40
48.	Dionda nubila	1	8	6	3	7	6	8	39
49.	Notropis spilopterus	2	8	4	4	8	4	8	38
50.	Pomoxis nigromaculatus	2	4	6	4	7	6	8	37
51.	Catostomus commersoni	1	2	7	4	8	6	8	36
52.	Micropterus dolomieui	1	1	6	2	8	8	8	34
53.	Fundulus kansae	4	8	4	0	8	0	8	32
54.	Esox lucius	1	2	5	3	7	6	7	31
55.	Salmo gairdneri	1	1	2	2	2	8	8	24
56.	Salmo trutta	1	1	1	1	1	8	8	21
57.	Dorosoma cepedianum	1	1	1	1	4	4	8	20

Table 6

Recommended Fish Species Previously

Used in Bioassay Research

Species	Lennon & Walker (1964)[249]	Hunn et. al. (1968)[142]	Mount (1968)[90]	Battelle (1971)[105]
Atlantic salmon *Salmo salar*				X
Threadfin shad *Dorsoma pentenense*			1	
Sea lamprey *Petromyzon marinus*				X
Rainbow trout *Salmo gairdneri*	X	X	1	X
Brown trout *Salmo trutta*		X		X
Brook trout *Salvelinus fontinalis*	X	X	1	X
Lake trout *Salvelinus namaychus*			2	
Coho salmon *Oncorhynchus kisutch*			2	X
Chinook salmon *Oncorhynchus tshawytscha*				X
Lake herring *Coregonus artedii*			2	
Mountain whitefish *Prosopium williamsoni*			2	
White sucker *Catostomus commersoni*	X	X	1	X
Bigmouth buffalo *Ictiobus bubalus*	X			
Goldfish *Carassius auratus*	X	X	1	X
Emerald shiner *Notropis atherinoides*			1	
Fathead minnow *Pimephales promelas*	X	X	1	X
Bluntnose minnow *Pimephales notatus*				X
Golden Shiner *Notemigonus chrysoleucas*	X			X

(Continued)

Note: 1 = primary list, all pollutants.
2 = special list, for selected pollutants.

Table 6 (Concluded)

Species	Lennon & Walker (1964)[249]	Hunn el. al. (1968)[142]	Mount (1968)[90]	Battelle (1971)[105]
Red-sided Shiner *Phoxinus phoxinus*				X
Creek chub *Semotilus atromaculatus*				X
Channel catfish *Ictalurus punctatus*	X	X	1	X
Black bullhead *Ictalurus melas*	X			X
Yellow bullhead *Ictalurus natalis*				X
Brown bullhead *Ictalurus nebulosus*	X	X		X
Mosquitofish *Gambusia affinis*				X
Guppy *Lebistes reticulatus*				X
White bass *Roccus chrysops*			1	
Yellow perch *Perca flavescens*	X	X	1	
Walleye *Styostedion vetreum*	X	X	2	X
Green sunfish *Lepomis cyanellus*	X	X		X
Bluegill *Lepomis macrochirus*	X	X	1	X
Pumpkinseed *Lepomis gibbosus*	X			X
Redear sunfish *Lepomis microlophus*				X
Longear sunfish *Lepomis megalotus*	X			
Smallmouth bass *Micropterus dolomieui*	X	X	2	X
Largemouth bass *Micropterus salmoides*		X	1	X
Northern pike *Esox lucius*	X	X	1	
American smelt *Osmerus mordax*			2	
Brook stickleback *Culaea inconstans*	X			
Threespine stickleback *Gasterosteus aculeatus*				X

Table 7

Species Recommended for Use in Bioassays

(References 88, 89, 90, 93, 142, 245, 248, 249)

Fish

- Mummichog (*Fundulus hereroclitus*)
- Winter flounder (*Pseudopleuronectes americanus*)
- Scup (*Stenotomus chrysops*)
- Atlantic salmon (*Salmo salar*)
- Striped bass (*Morone saxatilis*)
- Herring (*Clupea harengus*)
- Milkfish (*Chanos chanos*)
- Rainwater killfish (*Lucania parva*)
- Atlantic menhaden (*Brevoortia tyrannus*)
- Summer flounder (*Paralichthys dentatus*)
- Croaker (*Micropogon undulatus*)
- Striped mullet (*Mugil cephalus*)
- Sailfin molly (*Poecilia* (*Molliensia*) *latipinna*)
- Pinfish (*Lagodon rhomboides*)
- Southern flounder (*Paralichthys lethostigma*)
- Red snapper (*Lutjanus campechanus*)
- Gulf menhaden (*Brevoortia patronus*)
- Sheepshead minnow (*Cyprinodon variegatus*)
- Bay anchovy (*Anchoa* (*Anchoiella*) *mitchilli*)
- Pompano (*Trachinotus carolinus*)
- Chinook salmon (*Oncorhynchus tshawytscha*)
- Coho salmon (*Oncorhynchus kisutch*)
- English sole (*Parophrys vetulus*)
- Starry flounder (*Platichthys stellatus*)
- Shiner perch (*Cymatogaster aggregata*)
- Staghorn sculpin (*Leptocottus armatus*)
- Pacific sardine (*Sardinops caerulea*)
- Pink salmon (*Oncorhynchus gorbuscha*)
- Pacific herring (*Clupea pallasii*)
- Threespine stickleback (*Gasterosteus aculeatus*)
- Ling cod (*Ophiodon elongatus*)
- Mountain bass (*Kuhlia sandvicensis*)
- Anchovy (*Stolephorus purpureus*)

Crustacea

- Green crab (*Carcinides maenas*)
- Sand shrimp (*Crangon septemspinosa*)
- Lobster (*Homarus americanus*)
- Copepod (*Pseudocalanus minutus*)
- Barnacles (*Balanus* sp.)
- Blue crab (*Callinectes sapidus*)

(Continued)

Crustacea (Continued)
- Calico crab (Ovalipes ocellatus)
- Brown shrimp (Penaeus aztecus)
- White shrimp (P. setiferus)
- Pink shrimp (P. duorarum)
- Spiny lobster (Panulirus argus, P. interruptus, P. japonicus, P. pencillatus)
- Dungeness crab (Cancer magister)
- Shore crabs (Hemigrapsus sp.)
- Shrimp (Pandalus spp., Crangon spp.)
- King crab (Paralithoides camtschatica)
- Crab (Ranina serrata)
- Crab (Portunus sanquinolentus)
- Crab (Podophthalmus vigil)

Mollusca
- Eastern oyster (Crassostrea virginica)
- Soft-shell clam (Mya arenaria)
- Ribbed mussel (Volsella demissa)
- Quahog (Mercenaria mercenaria)
- Surf clam (Spisula solidissima)
- Bay scallop (Aequipecten irradians)
- Calico scallop (A. gibbus)
- Abalone (Haliotis spp.)
- Little neck clam (Protothaca staminea)
- Razor clam (Siliqua patula)
- Pismo clam (Tivela stultorum)
- Bay mussel (Mytilus edulis)
- Sea mussel (M. californianus)
- Pacific oyster (Crassostrea gigas)
- Ribbed limpet (Siphonaria normalis)
- Saltwater limpets (Helcioniscus exaratus, H. argentatus)
- Cone shells (Conus spp.)

Other Invertebrates
- Bloodworm (Glycera dibranchiata)
- Sandworms (Nereis virens, N. vexillosa)
- Sea urchin (Arbacia puntulata)
- Sea urchins (Lytechinus spp., Echinometra spp.)
- Sea urchin (Strongylorentrotus purpuratus)
- Sea anemone (Anthopleura elegantissima)

Thalloid Algae
- Sea moss (Chondrus crispus)
- Sea lettuce (Ulva spp.)

(Continued)

Table 7 (Concluded)

Thalloid Algae (Continued)
- Atlantic kelp (*Laminaria digitata*, *L. agardhii*)
- Red seaweed (*Gracilaria verrucosa*, *G. foliifera*)
- Bullwhip kelp (*Nereocystis luetkeana*)
- Giant kelp (*Macrocystis pyrifera*)

Unicellular Algae
- Local species of genera: *Cyclotella*, *Navicula*, *Nitzschia*, *Chaetoceros* and others

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Rosenberger, David R
Considerations in conducting bioassays / by David R. Rosenberger ... [et al.], Bioassay Laboratory, WAPORA, Inc., Charleston, Illinois. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1978.
127, [17] p. : 27 cm. (Technical report - U. S. Army Engineer Waterways Experiment Station ; D-78-23)
Prepared for Office, Chief of Engineers, U. S. Army, Washington, D. C., under Contract No. DACW 39-73-C-0134 (DMRP Work Unit No. 1D02)
References: p. 69-127.

1. Bioassay. 2. Freshwater fishes. 3. Toxicity. I. United States. Army. Corps of Engineers. II. Wapora, Inc. Bioassay Laboratory. III. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Technical report ; D-78-23.
TA7.W34 no.D-78-23